

USE OF ORGANOSOLV PROCESSES

RENEWABLE ENERGY DIVISION

PREPARED FOR:

Bioenergy Development Program
Renewable Energy Division
Coal and Alternative Energy Branch
Energy, Mines and Resources Canada
Ottawa, Canada
DSS Contract File No: 05SR.31926-4-5024
September 29, 1986

PREPARED BY:

Econotech Services Limited
852 Derwent Way
Annacis Business Park
New Westminster, British Columbia
V3M 5R1
Telephone: (604) 526-4221

SCIENTIFIC AUTHORITY:

Dr. R.P. Overend
Division of Energy Research and Development
National Research Council

Michie, R.I.C., Econotech Services Limited.

Use of Organosolv Processes. Report of Contract File No. 05SR.31926-4-5024. Renewable Energy Division; Energy, Mines and Resources Canada, Ottawa, Ontario. 1986. (159 pp)

Copies of this report may be obtained through:

Renewable Energy Division

Energy, Mines & Resources Canada

580 Booth Street

Ottawa, Ontario

K1A 0E4

CISTI Library

National Research Council

Bldg. M-55, Montreal Road

Ottawa, Ontario

K1A 0S2

Fermentation studies were carried out by B.C. Research, under sub-contract from Econotech Services Limited.

This report is distributed for informational purposes only and does not necessarily reflect the views of the Government of Canada nor constitute an endorsement of any commercial product or person. Neither Canada nor its ministers, officers, employees or agents makes any warranty in respect of this report or assumes any liability arising out of this report.

PREFACE

This is the final report on a project undertaken by Econotech Services Limited to investigate organosolv pulping of wood chips using alcohol-water mixtures. There has been a wide divergence in viewpoint as to the merits of organosolv pulping; the present study was intended to provide an independent base of technical data from which to make a realistic assessment of its commercial potential.

For technical reasons the investigation was essentially confined to single-stage pulping of aspen hardwood. With that limitation, however, this appears to have been the only study thus far concerned with isolation and utilization of not only the cellulose fibres, but the hemicellulose and lignin fractions as well. The results are likely to be of interest to government departments, companies, institutions and individuals involved in or concerned with the forest products industry.

ABSTRACT

This work was undertaken to provide an independent technical assessment of the potential of organosolv pulping to supply pulp, hemicellulose and lignin as usable raw materials. No such evaluation has appeared hitherto in the literature.

Aspen wood chips were pulped with methanol-water and ethanol-water mixtures at 190 deg C in a pilot scale digester. After the cook, the lignin was precipitated from the spent liquor by addition to acidified water and the separated liquor condensed in a flash evaporator. Material balance calculations were made from analytical data obtained throughout the processing.

The results reveal an inherent conflict between the requirements of high pulp strength and high recovery of hemicellulose. Highest strength was achieved at 66% pulp yield, but at that yield 50% of the hemicellulose, and 30% of the lignin, remained in the pulp. In addition, the liquors were found to be very toxic to fermentation bacteria and would need a purification treatment to allow fermentation of the hemicellulose sugars. These problems make it appear unlikely that single-stage alcohol pulping could ever be developed into a viable commercial process. Multi-stage cooking appears to offer greater potential and merits further investigation.

	Page
1. INTRODUCTION	1
1.1 Historical Development	1
1.2 Current Commercial Interest	4
1.2.1 Paszner Process	4
1.2.2 B.E.C. (APR) Process	5
1.2.3 MD Process	5
1.2.4 Battelle-Geneva Process	6
1.3 Summation	6
2. OBJECTIVES	10
3. METHOD	11
3.1 Summary of Procedure	11
3.2 General Experimental Details	13
3.2.1 Materials	13
3.2.2 Cooking Procedures	14
3.2.3 Isolation of Lignin from Spent Liquors	16
3.2.4 Concentration of Hemicellulose Solutions	17
3.2.5 Testing Procedures	18
4. RESULTS	23
4.1 Preliminary Single Stage Ethanol Cooks	23
4.1.1 Variation in pH During Cooking	23
4.1.2 Effect of Solvent Wash	24
4.1.3 Effect of Caustic Extraction	24
4.1.4 Material Balance	24
4.2 Higher Yield Methanol Cooks	25
4.2.1 Defibering	26
4.2.2 Physical Properties	27
4.2.3 Dissolving Pulp Evaluation	28
4.2.4 Isolation of Lignin and Hemicellulose	28
4.2.5 Carbohydrate Analysis	30

	Page
4.3 Cooks to Generate a Supply of Hemicellulose	31
4.3.1 Liquor Sampling	31
4.3.2 Processing of Liquor	32
4.3.3 Pulps	33
4.3.4 Analysis of Liquors	33
4.3.5 Recovery of Hemicellulose	34
4.3.6 Lignin	35
4.4 Short Duration Cooks	36
4.4.1 Cooking Conditions, Processing & Sampling	37
4.4.2 Pulps	38
4.4.3 Lignins	38
4.4.4 Analysis of Liquors	39
4.4.5 Pulp Yield and Liquor Retention	40
4.4.6 Liquor Left in the Digester	41
4.4.7 Recovery and Balance	42
4.5 Three-Stage BEC Cook	44
5. DISCUSSION	46
5.1 Recovery and Loss of Materials	46
5.1.1 Alcohol	46
5.1.2 Cellulose	47
5.1.3 Hemicellulose	48
5.1.4 Lignin	49
5.2 Fermentation of Hemicellulose Sugars	51
5.3 Paper Properties	53
5.4 Dissolving Pulp Properties	56
6. CONCLUSIONS	58
6.1 Specific Conclusions	58
6.2 Assessment	61
7. RECOMMENDATIONS	64

	Page
8. REFERENCES	66
9. APPENDICES	71
9.1 Appendix A - Tables of Results	71
9.2 Appendix B - Figures	98
9.3 Appendix C - B.C. Research Report, Phase I	106
9.4 Appendix D - B.C. Research Report, Phase II	137

EXECUTIVE SUMMARY

Organosolv pulping, which involves the removal of lignin from wood chips with an organic solvent, has trodden a long and somewhat controversial path since it was first proposed in the 1930's. The process offers a sulfur-free, and therefore environmentally attractive system, and also offers the prospect of recovering not only the cellulose pulp but also the lignin and hemicellulose fractions as usable raw materials. However, there are practical difficulties. Organosolv pulp is weaker than conventional kraft pulp, and handling flammable liquids at temperatures near 200 deg C and pressures up to 500 psig poses a major problem in production hardware. As a result, the North American pulp and paper industry has thus far shown little inclination to invest in any organosolv process.

Others have been less cautious. A process using ethanol is being developed at the pilot level by Biological Energy Corporation in the U.S., and L. Paszner at the University of B.C. has actively promoted single-stage methanol pulping for some years. Protagonists of organosolv systems have championed their cause with considerable optimism, and the divergence between their viewpoint and that of the more sceptical made it difficult to arrive at any objective evaluation of the process. The present work was undertaken

to provide an independent technical assessment of alcohol pulping, with the particular objectives of determining the optimum quality of pulp attainable, and how well the cellulose, hemicellulose and lignin could be separated. Forintek, Ottawa, was contracted by DSS to investigate the suitability of the hemicellulose for fermentation, and of the lignin for adhesive applications, and B.C. Research was sub-contracted by Econotech to carry out some additional fermentation studies.

Cooks to produce organosolv pulps were carried out in the Econotech high pressure digester. The wood used throughout the study was Trembling aspen, which is known to be particularly suited to alcohol pulping. It had been intended to study both a single-stage methanol process, and the BEC multi-stage ethanol process. However, it was not possible to simulate the BEC process effectively and at the same time produce the quantities of hemicellulose needed by Forintek for fermentation studies. Since the fermentation requirements were judged to be more important most of the cooks were carried out by the single-stage process.

The yield of pulp obtained depended (inversely) on the severity of the cooking conditions, particularly the cooking time and the acidity of the liquor. Pulp strength increased

with increasing yield, to a maximum at about 66% yield. At maximum, the strength was somewhat lower than that of kraft pulp made from the same wood. Nevertheless, there would probably be a market for the pulp as a filler in printing grades of paper.

Increasing yield results inevitably in an increased retention of hemicellulose and lignin by the pulp, and there is an inherent conflict between the requirements of achieving maximum strength together with maximum recovery of those components. At 66% yield, the pulp retained 50% of the original hemicellulose and 30% of the lignin, which would therefore be unavailable for any other end use. In addition, the lignin would have to be bleached, the cost of which could prove prohibitive.

The main obstacle to recovery of hemicellulose, for use as a raw material, is the retention by the pulp. Once extracted, there is a tendency towards degradation, particularly at high acidity, but from the data it appears that conditions could be adjusted to prevent this.

Toxic substances are formed, or extracted, during the cook which inhibit fermentation of the hemicellulose sugars, except at spent liquor concentrations so low as to be

commercially impractical. A solution to this problem must clearly be found if organosolv sugars are to be used as a fermentation substrate.

Lignin does not appear to be destroyed during the cook. However, recovery after precipitation was disappointing, scarcely exceeding 50% of the original. The reason for this was not clear, and could not be properly investigated because it was not possible to determine acid soluble lignin in presence of spent cooking liquor.

Methanol was not consumed in the cooking stage and should theoretically be available for 100% recovery. However, there are likely to be practical problems, depending on the recovery system used. A conservative estimate for ethanol losses expected to occur in the BEC process is 3 kg/tonne of pulp produced.

Bleaching to produce dissolving grades of pulp removed much less of the residual hemicellulose than expected, and less than normally required. In spite of that the pulp made a viscose solution which met laboratory test standards. The acetate solution was unacceptable because of very high haze and poor filterability, evidently due to the presence of the hemicellulose. It is not certain how much of the

hemicellulose would have to be removed to make the pulp suitable for acetate manufacture but the conditions of acidity required could well result in a pulp of unacceptably low viscosity.

The single-stage methanol process is beset by a number of major problems, probably the most serious of which is the inherent conflict between the requirements of high strength and high recovery of hemicellulose. It is recommended that no further work should be carried out on this process.

The BEC multi-stage process, with short cooking times, may offer a significant improvement over single stage pulping. Questions remaining unanswered concern how much of the lignin and hemicellulose can be extracted in the various stages, and how these can be separated efficiently. However, insofar as the viability of the process depends on being able to ferment the hemicellulose sugars the first priority should be to find ways of deactivating the toxicants.

An alternative possibility would be to use a multi-stage process with water instead of organic solvent in the first stage, to promote extraction of the hemicellulose. The lignin would then be extracted in one or more subsequent

stages using an organic solvent. In this way the hemicellulose and lignin should be obtained already substantially separated from each other, avoiding one of the major problems with the existing systems. It is recommended that experimental work should be undertaken to investigate the potential of water/alcohol pulping.

Organosolv pulping has been represented as a non-polluting process by which wood can be separated into its three major components, making each available as a raw material. However, in spite of the volume of literature in this field, the present investigation appears to have been the only attempt to quantify the system in terms of recovery of the pulp, hemicellulose and lignin components. As such it stands as an important technical reference work in any evaluation of the potential of the process studied. The study was essentially limited to single-stage cooking, and the data obtained indicate that a multi-stage process should be more effective. There is a need to carry out further work on multi-stage cooking, particularly with an aqueous liquor in the first stage. The analytical methods used in the present study were those commonly used in the industry. The data cast some doubt on the applicability of some of the methods to organosolv cooking liquors and before any further experimental work is carried out the methods need to be verified, and if necessary alternative methods developed.

1. INTRODUCTION

1.1 Historical Development

The chemical conversion of wood into pulp, suitable for papermaking and other applications, depends primarily on removal of the lignin bonding material, allowing separation of the individual fibres.

Traditionally, chemical pulping has been carried out by the kraft or sulfite processes, both of which modify the lignin to render it soluble in the pulping liquor. It has long been known that isolated lignin is soluble in organic solvents such as alcohol or acetone, and the possibility of using an organic solvent which might separate the lignin in a relatively unmodified form has been the subject of interest for many years.

Typical solvents used in earlier studies were: ethanol (1), phenol (2), alcohols (3), polyglycols (4), dioxane (5), butanol (6), glycol (7), glycerol (8) and dichloropropionaldehyde (9). The systems frequently employed a mixture of solvent and water with an acidic catalyst such as HCl (1,3,5,7,10), HNO₃ (11), chlorine (12), and SO₂ (13,14).

These early investigations on organosolv pulping did not lead to the development of any commercial scale process nor, apparently, was such development ever seriously considered at that time. The problems of trying to operate with flammable solvents at temperatures up to 200 deg C, coupled with the somewhat inferior properties of the pulps produced, made it appear unlikely that organosolv pulping could be operated as a commercially viable process.

However, in the 1960's an additional factor began to emerge, resulting from the growing concern for environmental considerations. Both the kraft and sulfite processes use sulfur, which is apt to find its way into the environment as malodorous mercaptans or noxious oxides. There was furthermore a growing tendency to regard traditional processes as wasteful since they utilized less than half of the wood, the remainder being burned or, worse, discharged with the effluent. The prospect of being able to pulp with a sulfur-free organic solvent which might at the same time enable recovery of not only the cellulose fibres but the hemicellulose and lignin fractions as well, sparked renewed interest in organosolv pulping.

Kleinert, who had been one of the early investigators, published a series of papers and obtained a patent on organosolv pulping of hardwoods and softwoods with aqueous ethanol (16-21). April and coworkers reported a number of investigations on the pulping of southern yellow pine with aqueous butanol or ethanol (22-26). Other publications relate to continuous pulping with aqueous ethanol or isopropanol and water (27) and with aqueous ethanol with aluminum catalyst (28).

Organosolv systems, particularly using aqueous alcohols, have most frequently been studied under acidic conditions. However, Nakano and coworkers carried out an extended series of investigations of cooking with alkaline methanol (29-35). Marton and Granzow (36) proposed a process based on a mixture of aqueous ethanol and NaOH, and McGee and April (37) applied this system to the cooking of southern yellow pine. Other alkaline processes involve cooking with aqueous alcohols and amines (38), methanol, NaOH and anthraquinone (39), aqueous ethanol at about pH 13 (40), and a high boiling solvent such as glycol or glycerol (41).

While it is true that one of the motivating forces behind the renewed interest in organosolv pulping lay in

the possibility of developing a sulfur-free pulping system, there has nevertheless been considerable interest in processes combining sulfite or sulfite-type liquors with aqueous alcohols (42-49). Rickey and Lovell (50) and Sarkanen (51) obtained patents for processes combining organic solvents with sulfides under alkaline conditions.

Some recent investigations have examined pulping systems using acetic acid (52), a mixture of aqueous ethylenediamine, ethanol and anthraquinone (53), and a mixture of water, acetic acid and ethyl acetate (54,55).

Prof. K.V. Sarkanen, at U. of Washington, Seattle, has been active in alcohol pulping for some years, but so far has published only one paper (56). However, he reviewed his more recent data with the author during a visit in July 1985.

1.2 Current Commercial Interest

1.2.1 Paszner Process

L. Paszner has for several years been extremely active in promoting organosolv pulping using aqueous methanol. He has several publications (57-60) and three patents (61-63). While commercial

interest in the process is at present uncertain it appears of sufficient prominence to merit inclusion in this section.

The Paszner process involves cooking wood chips at approximately 200 deg C with approximately 80:20 methanol-water in presence of an alkaline earth metal salt, particularly CaCl_2 , with or without the addition of an acid catalyst.

1.2.2 BEC (APR) Process

The Biological Energy Corporation Alcohol Pulping and Recovery process (64-66) is based on a patent obtained by Diebold, Cowan and Walsh (67). It involves pulping wood chips with 50% aqueous ethanol at about 200 deg C, in at least three stages, using successively cleaner liquor until in the final stage fresh liquor is used. Among the company's development facilities is a 25 pound per day pilot plant.

1.2.3 MD Process

A new company, MD Organocell, was formed by MD Papier in 1984, to produce organosolv pulp from wood (68). No details have been disclosed, but it

appears that the company's pilot plant facility, located in Munich, is operating a two-stage process using aqueous methanol (69).

1.2.4 Battelle-Geneva Phenol Process

In this process, lignocellulose is pulped with aqueous phenol at a somewhat elevated temperature, but below the temperature of furfural formation. On cooling the solution separates into two layers, one of which contains lignin dissolved in phenol and the other hemicellulose in water (70). The process apparently produces high purity cellulose suitable for dissolving grade pulps, but unsuitable for papermaking.

1.3 Summation

Published investigations in the organosolv area have treated the process simply as a pulping operation. Gomide (71) summarized technical results available in the literature for the ethanol system as follows:

- a) Higher pulping yield than with kraft, by about four percentage points, due to higher carbohydrate retention.
- b) Lower pulp strength than with kraft, in approximately the same range as soda or bisulfite pulps.

- c) Relatively short cooking cycles.
- d) Ethanol apparently not consumed during pulping and therefore available for total recovery.

Although the possibility of being able to utilize not only the cellulose fibres but also the lignin and hemicellulose components was one of the factors which generated interest in organosolv pulping, very little work seems to have been carried out to establish whether or not these components could be used. Two investigations have been reported dealing with the use of organosolv lignin as a wood glue (72) and as a petrochemical refiner feed (73). However, there appears to be no published work dealing with the fermentation of sugars derived from organosolv hemicelluloses for the production of commercially saleable solvents such as ethanol.

In 1984 a critical review of the organosolv pulping area was published in Pulp and Paper under the heading "Organosolv pulping process - boon or boondoggle?" (74). The review concluded "No one has yet synthesized an organosolv approach that blends technical, engineering, economic and environmental knowledge into a commercial package that can stand on its own merits." A vigorous

rebuttal followed, from E. Kendall Pye, President of BEC (75). On the other hand, Otto Forgacs, senior Vice-President R & D, MacMillan Bloedel Limited, added strong support for the position taken in the Pulp and Paper article (76). The foregoing sums up rather aptly the situation at the present time, namely a fierce antagonism on the part of those involved in organosolv development, and a substantial scepticism on the part of others, particularly those having to decide whether or not to invest development dollars in the process.

Any final evaluation of the system requires technical information, some of which is lacking altogether, and some of which is clouded by discrepancies between the findings of different investigators. For example, most investigators have found organosolv pulps to be inferior to kraft in strength whereas a recent BEC publication (67) claimed to have achieved equivalent strength.

Before attempting any economic evaluation of the organosolv pulping process, there are certain technical questions which have to be cleared up:

1. Just how good is the pulp produced?
2. What is the yield of recoverable lignin?
3. How can the lignin be utilized?
4. What is the yield of recoverable sugars?
5. Can the sugars be fermented, and at what yield of product?

The present investigation was undertaken to provide information relevant to the above questions. The program was directed towards pulping of aspen hardwood, because hardwoods are known to be more amenable to organosolv pulping than are softwoods (78), and there is a ready supply of aspen in Canada.

2. OBJECTIVES

The objectives of the present project were:

1. To carry out an independent and unbiased study of organosolv pulping of aspen hardwood, particularly by a single-stage methanol process and the BEC ethanol process, to establish:
 - a) the quality of pulp
 - b) the yield of sugars
 - c) the yield of ligninattainable under various processing conditions.
2. To obtain mass balance data, within the scope of the available analyses.
3. To produce a supply of sugars for fermentation studies, and lignin for adhesive evaluation.

3. METHOD

3.1 Summary of Procedure

Aspen chips were cooked by a single-stage process, and also by a multi-stage process based on the BEC system. After cooking the pulps were evaluated, and in later trials the pulps, lignins and various liquors analyzed to determine the distribution of the various components.

The single-stage Paszner process involves cooking wood chips at approximately 200 deg C with approximately 80:20 methanol-water, in presence of an alkaline earth metal salt, particularly CaCl_2 , with or without the addition of an acid catalyst, and preferably at a pressure in excess of that developed by the vapour pressure of the liquor.

The requirement of excess pressure is the cornerstone of Paszner's third patent (63), and involves pressures in the neighbourhood of 300 atmospheres (4,400 psig). Such pressures are scarcely practicable in an industrial pulping process, and are beyond the capacity of the digester at Econotech by an order of magnitude. All cooks were carried out at the vapour pressure of the liquor (about 350 psig), but otherwise under conditions basically similar to those specified in Paszner's publications (57,60-63).

In the BEC process, wood chips are pulped with approximately 50% aqueous ethanol at about 200 deg C in at least three stages, using successively cleaner liquor until in the final stage fresh liquor is used. According to the patent (68) no catalyst is used.

For the present investigation the BEC process was simulated by first preparing spent liquor and using this to make up liquor corresponding to the first two stages of a 3-stage process. Each stage was carried out basically by the same procedure used for the single-stage cooks. It was recognized that this system was not ideal since the cooking liquor had to be discharged between successive stages which allowed the chips to cool down and dry out to some extent. Use of the digester side pot, which can be isolated from the main digester, would have minimized the problem. Unfortunately, the side pot holds only 400 g of chips and this was insufficient to generate the quantities of hemicellulose required by Forintek for fermentation studies.

Rather than proceed with multi-stage cooking in the larger digester, under conditions which would have cast some doubt on the validity of the results, it was

decided to concentrate the subsequent trials on the single-stage process.

3.2 General Experimental Details

This section records experimental details relevant to all, or most, of the cooks. Specific details for individual cooks or cook series are included in the Results, Section 4.

3.2.1 Materials

The wood used was Trembling aspen obtained from the O'Chiese Block, Brazeau Timber Development, Alberta Foothills, elevation 1,000 m. The age ranged from 24 to 53 with an average of 38 years, the log diameter from 14 to 25, average 18 cm, and the juvenile wood, calculated from the diameter at 14 years, from 4 to 26, average 14%. The knot content was normal for the species and the logs were essentially free from rot, pitch pockets and reaction wood. The logs were shipped to Econotech immediately after logging and chipped on receipt, using a 6 knife, 40 inch CAE chipper. The chips were stored in the dark at 3 deg C, in airtight bags. Analytical details are given in Table 3.1.

Sample weights are expressed on an oven dry (o.d.) basis. Thus 3 kg o.d. of wood chips means 3 kg of wood substance, irrespective of the amount of water present.

The ethanol used was Commercial Alcohols D.A.G. 2-A having the following composition:

	<u>Percent v/v</u>
Ethyl alcohol	80.7
Methyl alcohol	14.3
Methyl ethyl ketone	0.5
Ethyl acetate	0.2
Water	Balance

The methanol was supplied by Harrisons & Crosfield at an assay of not less than 99.5%.

The composition of the cooking liquor is specified in terms of make up by volume. Thus 80:20 ethanol-water refers to 80 volumes of ethanol mixed with 20 volumes of water.

3.2.2 Cooking Procedures

The aspen chips, contained in a wire basket, were placed in the 30 litre stainless steel organosolv

digester. Pulping liquor was introduced at the bottom, circulated with an external pump and heated with an external steam heat exchanger. At about 90 deg C gases were vented to remove air and the digester was then heated to 190 deg C for the required cooking time. At the end of the cook liquor was "blown", that is was forced under its own vapour pressure into a 33 gallon flash tank and the vapours discharged outside the building.

The 30 litre digester accommodated 3 kg of chips. For smaller samples the digester was equipped with a 3 litre side pot, which held 400 g of chips. In using the side pot, the liquor was first preheated to 190 deg C in the main digester and then circulated through the pot. In this way cooking was carried out under practically isothermal conditions.

The cooked chips were defibered, where possible, using a Lightnin Mixer (a pilot scale propellor-type stirrer). Higher yield materials could not be defibered in this way and so were given one pass through a Sprout-Waldron single disc refiner at 0.025" clearance. After defibering the pulp was

screened with a 10 cut Valley flat screen, washed and stored at 25% consistency (75% water) at 3 deg C.

Caustic extractions were carried out in plastic bags at 10% consistency, following which the pulps were washed well with hot water (50 deg C) and finally cold water.

3.2.3 Isolation of Lignin from Spent Liquors

In an early trial (Cook 5) the spent liquor was simply evaporated from a volume of about 15 litres to 1.4 litres. This had the effect of precipitating most the lignin, but in doing so contaminated the evaporation apparatus, particularly the flash evaporator, and the method was judged to be unsatisfactory.

In a series of bench trials it was found that the lignin could be effectively precipitated from solution by slow addition of the spent liquor to one or two volumes of water acidified with HCl or H₂SO₄ to give a final pH of about 2. This method was used in the subsequent pilot scale trials. Following precipitation, the lignin and supernatant

liquor were allowed to stand overnight after which the lignin was removed by filtration using Whatman No. 2 filter paper.

3.2.4 Concentration of Hemicellulose Solutions

The filtrates from the precipitated lignin were evaporated using a flash evaporator operating under vacuum. The evaporator took a charge of 4 litres of liquor which was reduced to 1.5 litres in approximately 45 minutes. The total volume of liquor at the start was typically 40 litres so that 10 evaporation batches were required in the first stage to reduce the volume to 15 litres; 4 batches in the second stage for an evaporated volume of 5.6 litres; and so on until a final total volume of about 1 litre was reached. Generally, 4 stages were required so that each batch of liquor was heated for a total of 3 hours at a temperature increasing from about 50 to about 60 deg C, as the alcohol was removed.

Sugars are known to exhibit maximum stability under acid conditions (77). Accordingly, the liquors were kept acid during flash evaporation. The aim was to keep the pH in the range 2-4 but in some of

the earlier production runs the pH fell somewhat below this range.

There was some concern in the early stages about the level of alcohol which might remain after the evaporation. However, it was established in a pilot experiment that 93% evaporation of a methanol-water solution initially of concentration 80:20 v/v, reduced the alcohol concentration to less than 0.5% (Table 3.2). It was therefore concluded that residual alcohol would not cause any problem.

During the various evaporation stages there was some deposition of lignin in the equipment. This was removed from time to time by flushing with alcohol.

3.2.5 Testing Procedures

Testing and analyses were carried out by standard TAPPI procedures with the following exceptions:

- a) PFI refining, for paper handsheet properties, was carried out with 24 g pulp, 10% consistency and 0.02 mm clearance (CPPA procedure).

- b) S18 (pulp solubility in 18% NaOH) was measured at 20 deg rather than 25 deg C.
- c) The TAPPI method for sugar determination by gas chromatography, using the derived alditol acetates, gave figures for mannan which were consistently too high by about 0.5% points. The problem was solved by the use of an alternative acetylation method. Thus 350 mg of the sugar alditols were acetylated with 5 ml pyridine and 5 ml acetic anhydride for a minimum of 10 hours under reflux. The solution was evaporated to dryness on a rotary evaporator and any residual acetic acid removed by adding 1 ml portions of water and again evaporating to dryness. The dried extract was then taken up in methylene chloride.
- d) Analysis of sugars in the pulping liquors was carried out as with the pulp but without the primary hydrolysis in 72% H₂SO₄.

A problem became apparent in determining arabinan, one of the hemicelluloses, in that on certain occasions analysis indicated more arabinan in the

cooking liquor than in the original wood. In the GC determination the arabinose peaks were very sharp, with no sign of interference from another compound; and it seems most unlikely that arabinose could have been formed from one of the other carbohydrates under the acidic conditions of the processing. The problem remained unresolved, but since there was only 0.5% arabinan in the wood, the safest course appeared to ignore it altogether. Analytical data for arabinan have not therefore been reported for most of the trials.

The concentration of alcohol in the pulping liquors was initially determined by gas chromatography. However, repeat analyses were not always in very good agreement, differing in some instances by as much as 20%. It was found much more satisfactory to determine the water by Karl Fischer titration and calculate the alcohol content.

It was hoped to be able to determine the lignin content of the pulping liquors simply by UV absorption at 205 nm. A method was worked out which avoided interference from any alcohol present. However, when compared with Klason lignin

determination, the UV method gave values for lignin content which were, in the main, too high - by as much as 40% in one instance. It was concluded that some other component in the system, perhaps a sugar degradation product, was interfering with the determination, rendering it of no practical value. Permanganate, which reacts quantitatively with lignin and is used to determine lignin present in pulps, could not be used with the liquors because of interference from reducing sugars present, which also react with permanganate. There was therefore no alternative but to rely on Klason determinations of lignin in the cooking liquors.

In the standard TAPPI procedure for determining lignin in pulp it is customary to report the acid insoluble (Klason) lignin and also the acid soluble lignin, to give the total lignin content. The acid soluble fraction, determined by UV absorption at 205 nm, constitutes typically about 10% of the total in most pulps. This was found to be the case with pulps produced in the present work. However, in determining the lignin content of cooking liquors there was evidence of the same interference which had rendered the UV absorption method

impractical for the total determination, as detailed above. In some instances the "soluble lignin: was actually much greater than the Klason, and the total indicated more lignin than in the original wood chips. Figures for "soluble lignin" have therefore not been reported for the liquors.

4. RESULTS

Single-stage cooks were carried out in groups or series, and the conditions used in a series were to a large extent determined according to information gained in the previous trials. It is therefore appropriate to consider the various series in chronological sequence.

4.1 Preliminary Single-Stage Ethanol Cooks

Cooking conditions and pulp properties are shown in Table 4.1, included in which are data for a kraft pulp made from Batch 1 of the aspen chips and a sulfite pulp made from Batch 3. The sulfite pulp was cooked with magnesium bisulfite at pH 4.1-3.4. Physical properties of handsheets made from the pulps, listed in Table 4.2, will be discussed subsequently, in conjunction with other data obtained with pulps cooked to higher yields (Section 4.2.2).

4.1.1 Variation in pH During Cooking

Figure 4.1 shows the variation of pH with cooking time for cooks 2-5. There was a consistent pattern of behaviour in that the pH rose rapidly initially and then gradually fell to a final figure of about 4.1. The starting pH of about 3.2 reflected the presence of HCl (the pH of 0.05M CaCl₂ is about 5.4).

4.1.2 Effect of Solvent Wash

The ethanol wash following defibration removed over half the residual lignin, indicated by the decrease in kappa number (proportional to lignin content). The use of acetone instead of ethanol removed approximately the same quantity of lignin, showing this solvent to have no obvious advantage. Acetone was not used in any of the subsequent trials.

4.1.3 Effect of Caustic Extraction

Caustic extraction of the alcohol washed pulp from Cook 3 lowered the lignin content by as much as 40%, as shown by the decrease in kappa number (Table 4.3). The S18 (solubility in 18% NaOH) showed a modest decrease, taken to indicate removal of some of the hemicellulose from the pulp. This most probably accounts for the decrease in tensile and burst strength and increase in tear strength in the extracted pulps (Table 4.4).

4.1.4 Material Balance

An approximate material balance, ignoring possible losses on washing, is shown in Table 4.5 for Cook 5. Only 51% of the original hemicellulose was found in the pulp, isolated lignin and spent

liquor, and of that 51% approximately two-thirds remained in the pulp. This was an early indication of the problems associated with trying to isolate hemicellulose using the organosolv system. It appeared that quite extensive sugar degradation had taken place since, of the solids in the liquor, only 40% could be accounted for by analysis.

4.2 Higher Yield Methanol Cooks

Data obtained in the preliminary single-stage cooks indicated a continuous increase in pulp strength with increasing pulp yield, over the yield range 53-61%. It was of interest to establish just how high the yield could be taken without strength loss, so a series of cooks was carried out under milder conditions than used in the initial trials, to produce pulps at higher yield.

The conditions chosen were based on Paszner's published findings (58), using 80:20 methanol-water with $MgSO_4$ catalyst. At that time the need to supply Forintek with an immediate and continuing supply of hemicellulose for fermentation studies had not been made clear, and it seemed needlessly wasteful to cook 3 kg of chips at a time when only a small fraction of that was needed for analysis and testing. Accordingly, the cooks were

carried out in the digester side pot, using a chip charge of 400 g o.d. This offered an additional advantage of being able to cook under almost isothermal conditions.

Cooking conditions are given in Table 4.6 and analyses of the pulps in table 4.7. In the first three cooks (10-12), intended to provide the highest yields, the $MgSO_4$ concentration was held at 0.024 molar, and thereafter increased to 0.05 molar. Unfortunately, in the subsequent cooks a problem developed with the electrical heating system with the result that the side pot took longer to attain the 190 deg C cooking temperature than should have been the case (with Cook 16 the temperature only reached 186 deg C). Thus in trying to correlate yield or kappa number with cooking time there is some experimental variation between Cooks 10-12 and Cooks 13-16. However, the yield-kappa number relationship, shown in Figure 4.2, is reasonably consistent over the whole range of cooks.

4.2.1 Defibering

In the preliminary trials the pulps had been defibered in water and then washed with alcohol to remove any soluble lignin. It appeared that the

process of lignin removal ought to be more efficient if defibering could be carried out in alcohol rather than water and an attempt was made to do this. However, with these rather high yield pulps it was not possible to defiber fully using the Lightnin Mixer, and the Sprout-Waldron refiner plugged up due to rapid evaporation of the methanol used. Therefore, the procedure adopted was to defiber the pulps to whatever extent possible in 80:20 methanol-water using the Lightnin, at which point the pulp yield was determined, and then defiber fully in water using the refiner.

4.2.2 Physical Properties

Physical properties of pulp handsheets are listed in Table 4.8. The variation in strength with yield is shown in Figure 4.3 (tensile), Figure 4.4 (burst) and Figure 4.5 (tear), for the present pulp series and for the lower yield pulps made in the preliminary cooks. Although the two series were carried out under somewhat different conditions the strength-yield relationship appears consistent over the whole yield range, with a maximum in tensile and burst strengths at 63% yield and in tear strength at 70% yield. Data for the kraft and

sulfite reference pulps are included in the figures for comparison. Also included are results obtained with the 3-stage pulp, described subsequently in Section 4.5.

4.2.3 Dissolving Pulp Evaluation

Dissolving pulp is chemical pulp of very high purity, used in the manufacture of viscose and acetate rayon, cellophane and other products. There is not a large market for this material - the projected production for 1986 is only about 2% of the pulp total for North America, based on production capacity (79). Nevertheless, it was considered worth carrying out at least one evaluation of organosolv pulp for dissolving applications, particularly since Econotech has a high level of technical expertise in this area.

For the evaluation, the pulps produced in Cooks 10, 13, 14 and 15 were combined and the resulting blend bleached to remove the lignin and some of the hemicellulose. Two slightly different bleach sequences were used to produce pulps suitable for viscose and acetate. Details of the sequences are given in Table 4.9, and properties of the pulps produced in Table 4.10.

The pulps were evaluated using Econotech standard test procedures for dissolving pulps. The pulp bleached to viscose grade, Table 4.11, was found to meet the laboratory test standards for that end use, as shown by the comparison with data for typical commercial viscose grade pulps included in the table. On the other hand, the acetate pulp, Table 4.12, was unacceptable, having low reactivity, very high haze and colour and very poor filterability. These deficiencies are probably due to the high level of hemicellulose present. For acetate grade pulps made by the kraft or sulfite processes the xylan content should be no greater than about 1.5%, whereas there was 11.3% xylan in the pulp tested.

4.2.4 Isolation of Lignin and Hemicellulose

The spent liquors from Cooks 10-12 were combined, and the liquors from Cooks 13-16 combined. In each case the combined liquors were added to 2.5 volumes of water acidified to pH 2 with HCl to precipitate the lignin. The liquors were then evaporated by distillation under vacuum to give "evaporated liquor" containing less than 3% methanol, which at the time was thought to be the requirement for fermentation studies.

Subsequently it became clear that for fermentation a hemicellulose content in the liquor of at least 10% was required. Accordingly, the two evaporated liquors were combined and reduced further to a final volume of approximately 1 litre, using the flash evaporator as described in Section 3.2.4. As it turned out, the required level of hemicellulose was not attained. However, it was decided not to continue the evaporation because an incipient odour of caramel indicated the sugars were starting to degrade.

4.2.5 Carbohydrate Analysis

The present series of trials was undertaken primarily to assess the physical properties of single-stage pulps at high yield. However, carbohydrate analyses were carried out on the pulps and on the combined liquors and lignins from Cooks 10-12 and 13-16 (Tables 4.7 and 4.13). This enables an estimate to be made of the carbohydrate distribution between pulp, lignin and liquor for each of those two groups of cooks.

The results (Table 4.14) show that at these higher yields, 43-49% of the hemicellulose remained with

the pulp, with only 12-16% in the liquor. As in the preliminary trials, very little of the hemicellulose was found in the lignin fraction. Overall it was possible to account for, at most, only 67% of the original hemicellulose (Cooks 10-12), again indicating that substantial carbohydrate degradation had taken place.

4.3 Cooks to Generate a Supply of Hemicellulose

Given the requirement for providing Forintek with a continuing supply of hemicellulose for fermentation studies, the cooking program was resumed using 3 kg charges of chips in the main digester. An acid catalyst was added to promote extraction of hemicellulose from the wood, H_2SO_4 being used rather than HCl to reduce the possibility of corrosion in the digester. Cooking conditions are given in Table 4.15 for the 10 cooks carried out in this series.

4.3.1 Liquor Sampling

An outlet, leading from the digester circuit through a cooling coil, enabled samples of liquor to be removed during the cooking cycle. The coil cooled the liquor to approximately 40 deg C, well below the boiling point of the liquor at

atmospheric pressure (about 70 deg C) but still high enough to give some concern regarding possible losses of methanol due to evaporation. However, it was found in an initial trial that passing the liquor sample into cold 80:20 methanol, rather than into an empty specimen bottle, had no effect on the calculated methanol content of the liquor, from which it was concluded that evaporation was not a significant problem.

Samples (200 ml) were taken 5 minutes after reaching the 190 deg C cooking temperature, and 300 ml samples taken after 30 minutes and at 30 minute intervals thereafter to the end of the cook.

4.3.2 Processing of Liquor

To precipitate the lignin the spent liquors were added slowly to water containing 1.5 g conc. HCl/litre. For Cooks 17-20, two volumes of acidified water were used. For Cooks 21-26, this was reduced to 1.5 volumes to lessen the time required in the subsequent evaporation.

4.3.3 Pulps

The composition and properties of the pulps are shown in Table 4.16. The pulps suffered extensive degradation as a result of the somewhat low pH of the cooking liquors, and the pulp viscosities were too low to make it worth testing handsheets. The physical properties of the pulps were therefore not determined in this series.

4.3.4 Analysis of Liquors

Samples taken at 30 minute intervals during the cooks were analyzed for carbohydrates, total solids and methanol content, the results are presented in Table 4.17, showing the total weight of each component present at the time of sampling, to facilitate comparison between the various stages.

Methanol was not consumed during the cooks, as shown by the figures for methanol content of the liquors at various stages. The mean methanol content over all of the data was 76.3% w/w, which was not significantly different from that of the starting liquor, consisting of 80:20 v/v methanol-water, with a methanol content of 76.0% w/w.

Extraction of the hemicellulose from the wood chips did not proceed as expected. Rather than a continual extraction resulting in a continual buildup in the liquor, the hemicellulose was apparently extracted very rapidly in the early stages of the cook to reach a more or less stable plateau. There was some variation between the different hemicelluloses but overall the behaviour was essentially that of the main component, xylan.

At no stage in any of the cooks did the level of xylan in the liquor exceed approximately one-third of that in the original wood chips. It seemed that this might be the result of an equilibrium between dissolution and redeposition of the hemicellulose, although it was recognized that it could also be due to a balance between the rates of extraction and degradation.

4.3.5 Recovery of Hemicellulose

The distribution of hemicellulose between the cooking liquor, evaporated liquor and pulp is shown in table 4.18, in each case the values being expressed as a percentage of the original hemicellulose in the wood chips. The efficiency of

hemicellulose recovery was disappointingly low. In no case was the recovery in the evaporated liquor greater than 25%, and with Cook 17 it was as low as 2%. The overall recovery, in the evaporated liquor, pulp and liquor samples, did not exceed 36%. This appeared due primarily to a substantial loss in hemicellulose during the cook itself, with a possible additional loss during subsequent processing and evaporation.

As a check on the sampling procedure, the distribution of total solids was also calculated. The results, Table 4.19, show that within experimental error all of the solids were accounted for in the cooking liquors and the pulps. The validity of the sampling procedure was thus confirmed, showing that there had indeed been substantial loss in hemicellulose during the cooking stage.

4.3.6 Lignin

Primary interest in the present series of trials centred on the fate of the hemicellulose. However, in two instances Klason lignin determinations were carried out on samples of cooking liquor. The

result, table 4.20, show that in those two cases the lignin in the liquor, together with the lignin remaining with the pulp, accounted for 95% of the original lignin in the wood.

Recovery of the lignin was, however, substantially less quantitative. The data in table 4.21 show, in general, less than 50% of the original lignin was recovered by precipitation from the methanol solution. The reason for this was not clear, and it appeared that additional experimental data would be required to provide an explanation.

4.4 Short Duration Cooks

The previous series of cooks demonstrated clearly that cooking for extended periods was ineffective in increasing the extraction of hemicellulose from the wood chips. Viewing the data as a whole, it appeared that maximum extraction was already achieved within the first 30 minutes after reaching the cooking temperature of 190 deg C. The present cooks were therefore planned to study the early stages of cooking, particularly at times before attainment of cooking temperature. In some trials the acidity was increased in an attempt to promote extraction of hemicellulose in the short cooking time allotted.

It was hoped to obtain information bearing on two questions which remained unanswered from the previous trials, namely, why the recovery of hemicellulose was so low, and why the recovery of lignin was so low. Analyses were therefore carried out at all stages of the cooks and the subsequent liquor processing. In contrast to all other cook series carried out in this project, the cooking time here refers to the time from start up, when heat was first applied to the digester.

4.4.1 Cooking Conditions, Processing and Sampling

Cooking conditions, Table 4.22, were basically the same as in the previous series, with 80:20 methanol-water at a liquor ratio of 9.3:1, but with acidity varying between 0.01 and 0.05 molar H_2SO_4 . After cooking, lignin was precipitated by adding the blown liquor to 2 volumes of water containing 1.5 g/l H_2SO_4 (Cooks 29 and 30) or 1 volume of water containing 3 g/l HCl (Cook 31). Samples (400 ml) were taken at various stages of the cooks and from the blown liquor. Thereafter sampling was as follows:

Filtered liquor	500 ml
After 1st evaporation	200 ml
After 2nd evaporation	100 ml
After 3rd evaporation	50 ml
Final liquor	50 ml

The liquor was adjusted to pH 2 at each evaporation stage.

4.4.2 Pulps

Pulp analyses and properties are given in Table 4.23. In spite of the short duration of the cooks, the pulp viscosities were still not high enough to make it worth determining handsheet properties. Evidently the cellulose had suffered quite extensive hydrolysis as a result of the acidity in the cooking liquor.

4.4.3 Lignins

Analyses of the recovered lignins are shown in Table 4.24. Again, very little of the hemicellulose was retained by the lignin in the process of precipitation.

4.4.4 Analysis of Liquors

Results of analyses carried out on all the various liquor samples are shown in Table 4.25. As before, the original determinations in g/litre have been recalculated to show the total weight of each component present at each sampling, to facilitate comparison between the various stages.

The behaviour in this series was very much influenced by the acidity of the cooking liquor. As the acidity was increased, delignification increased, and hydrolysis of the cellulose increased dramatically, indicated by the increase in soluble glucan present. With the highest acidity (Cook 29) the fibrous structure of the pulp was more or less completely destroyed, the resulting product being more like mulligatawny soup than pulp. Increasing acidity also apparently promoted liberation of the hemicellulose, and then also its subsequent degradation. This was particularly marked in Cook 29, which exhibited the highest level of hemicellulose (xylan) at the 30 minute interval, and the lowest level at 60 minutes.

One other point emerging from the raw data is that the total weights of hemicellulose, glucan and lignin scarcely accounted for more than 75% of the total solids (Table 4.2.6). Some of the deficiency might be accounted for by acid soluble lignin, which could not be determined in the liquors. However, it seems probable that the difference is mainly due to solid degradation products, presumably from the hemicellulose, which were not identified in the present work.

4.4.5 Pulp Yield and Liquor Retention

In the cooks described in Section 4.3 it was shown that quantitative agreement existed between the total solids in the liquor at the end of the cook, the solids taken in sampling and the pulp yield (Table 4.19). In applying the same analysis to the present cooks, similar agreement was obtained with Cook 31. However, with Cook 30 only 93.2% of the original solids was accounted for; and with Cook 29 a balance could not be obtained because the pulp was so degraded it was not properly retained in the wire basket and so could not be recovered quantitatively. Since the Cook 30 pulp was also quite degraded, evidenced by the low viscosity, it

seems quite likely that quantitative recovery was not achieved with that pulp either. Very small particles of degraded pulp, which could pass easily through the containing basket, would likely remain suspended in the liquor until the digester was blown. They would then settle out and thus not be included in the liquor total solids, which would account for the discrepancy in solids balance.

There appears no reason why the solids balance found previously should not apply to the present cooks, and assuming that to be the case it is possible to calculate a corrected pulp yield for Cook 30, and a pulp yield for Cook 29. The relevant figures are given in Table 4.27.

For a material balance it is necessary to know how much liquor is retained by the chips after blowing the digester. The wet weight of chips was recorded, but these figures were discarded as meaningless because of the extensive evaporation from the chips during the 5 minute period of vapour flashing following discharge of the spent liquor.

An alternative method was offered by determining the amount of retained solids from the difference between the chip yield and pulp yield, and calculating the retained liquor volume from the concentration of solids in the liquor at the end of the cook (before blowing). The results of the calculations are shown in Table 4.28. With both Cooks 30 and 31 there was good agreement in the ratio of liquor retained to pulp weight. Using that value, 3.7 litres/kg, the liquor retained by the pulp in Cook 29 was calculated to be 3.6 litres.

4.4.6 Liquor Left in the Digester

After blowing the cook a certain amount of liquor was left in the digester assembly and associated piping. It was not possible to determine the volume of this liquor directly. However, by subtracting the solids, in the recovered spent liquor and in the liquor retained by the chips, from the total solids in the digester liquor at the cook end, it was possible to calculate that approximately 3 litres of liquor must have been left in the digester assembly.

4.4.7 Recovery and Balance

Table 4.29 shows the disposition of hemicellulose at various stages of the cooks and liquor processing, with the data expressed as a percentage of the hemicellulose in the original wood chips.

The most important observations concern the recovery of hemicellulose in the final evaporated liquor. Recovery increased markedly with decreasing acidity in the cooking liquor, reaching a high of 44% in Cook 31 (0.01M H_2SO_4). There was substantial loss of hemicellulose during cooking at the higher acidities and although degradation products were not identified it seems reasonably certain that the loss was indeed due to hemicellulose degradation. Viewing the data overall, relatively little hemicellulose was lost in the subsequent stages of processing, including the evaporation.

The amount of hemicellulose retained by the pulp decreased markedly with increasing acidity in the liquor. As found previously, the precipitated lignin retained very little of the hemicellulose.

A corresponding recovery and balance for Klason lignin is shown in Table 4.30. The most significant losses occurred in the process of precipitation and filtration, reaching a high of 20% in the case of Cook 31.

Table 4.31 shows recovery and balance for total solids. The losses at the various stages were within the limits normally encountered in pilot scale operations.

4.5 Three-Stage BEC Cook

A 3-stage cook based on the BEC process was carried out at a fairly early stage in the project. Spent liquor was first generated in Cook 6, carried out essentially by the single-stage procedure but using 50:50 ethanol-water with no catalyst. In blowing the digester, the alcohol content fell to 37% and was readjusted to the starting concentration by addition of further ethanol, to give a base spent liquor containing 28 g/L total solids.

In carrying out the 3-stage cook (Cook 7) cooking liquor was prepared using a mixture of the base spent liquor and ethanol-water, as indicated in Table 4.32. After

each stage the liquor was discharged from the digester and liquor for the next stage pumped in. In all other respects the cooks were carried out as with the single-stage trials. Details of the cooking conditions, and analyses of the pulps are included in Table 4.32. Physical properties of handsheets are shown in Table 4.33.

Both the preparatory and 3-stage pulps differed from those made using the single-stage catalyzed process in that the viscosity and strength were higher. The strength improvement increased in the order: burst - tensile - tear, as shown by the data plots in Figures 4.3-4.5. In the case of burst strength the improvement was barely significant, while the tear strength increased to the point where it almost matched that of the kraft pulp.

Extraction of hemicellulose from the pulp did not seem to be particularly favoured in the 3-stage process. The pulp retained significantly more xylan and mannan than did the single-stage pulp obtained in Cook 18 at essentially the same yield. However, the difference could be accounted for by the higher level of acidity in the single-stage cook.

5. DISCUSSION

5.1 Recovery and Loss of Materials

Material recovery in a chemical process depends basically on three factors:

1. The chemical stability of the materials under the processing conditions.
2. Intrinsic limitations, such as residual solubility in the case of a precipitation stage.
3. The efficiency with which the recovery plant can be made to operate.

In the present project it was obviously not possible to address Factor 3 directly. However, the investigation did produce a considerable amount of information bearing on problems likely to be associated with material recovery on a production scale.

5.1.1 Alcohol

Analyses carried out on the liquors in Cooks 17-26 showed that the methanol concentration remained constant throughout the course of those cooks (Table 4.17). This would appear convincing confirmation of Gomide's assessment (71) and Sarkanen's view (78) that alcohol is not consumed during organosolv pulping.

Whether or not the alcohol can be recovered quantitatively and economically is another matter. In the BEC "Alcohol Pulping and Recovery Process" alcohol rich vapours, from steam stripping of the pulp and from flash evaporation of the liquor, are recovered by condensation. No figures have been published concerning the efficiency with which the recovery can be carried out. Also, there is an open question about the amount of steam required to strip the alcohol from the pulp; but it would seem, a priori, that a law of diminishing returns dictates that some alcohol would have to be left behind. As alcohol is removed from the liquor the lignin precipitates out, and is finally separated as a cake at 60% solids (64), the remaining 40% being liquor. Even if the alcohol content of the liquor at that stage were as low as 1%, there would be an inevitable and continuing loss due to the liquor retained amounting to about 3 kg per tonne of pulp produced.

5.1.2 Cellulose

Under conditions of high acidity, particularly Cook 29, there was considerable loss of cellulosic material from the fibres, appearing as glucan in

the cooking liquor. However, under the milder conditions appropriate for the production of paper grade pulp the loss was insignificant (0.2% in Cook 5). Quantitative recovery of the pulp presented no special problems not commonly encountered in pulp processing.

5.1.3 Hemicellulose

The most revealing information concerning the behaviour of hemicellulose during cooking was obtained in Cooks 29-31. Clearly the extraction and degradation of hemicellulose, particularly the major component xylan, are both very pH sensitive. At the lowest pH (1.9 in Cook 29) the xylan was extracted very rapidly at the start and thereafter degraded very rapidly (Table 4.25). At the highest pH (3.3 in Cook 31) extraction took place more slowly but the xylan was scarcely degraded at all, according to the balance shown in Table 4.29. After cooking the hemicellulose remained quite stable, with scarcely any loss during the process of liquor evaporation (Table 4.29).

It thus appears that if the hemicellulose can be extracted from the pulp intact there should be no

problem in recovering it more or less completely in subsequent processing. According to Table 4.29 a substantial amount was retained in liquor held by the chips, and it was shown in Section 4.4.6 that a significant amount was left in the digester system; but this material could certainly be recovered, by washing the chips and flushing the digester.

The initial extraction from the pulp does appear to present some difficulty, however, this question will be discussed further in Section 5.3.

5.1.4 Lignin

The data obtained in Cooks 29-31 show that the rate of extraction of lignin from the wood chips increased as the acidity of the pulping liquor increased. However, unlike the situation with hemicellulose, there was no significant loss of lignin during cooking, even under the very severe conditions of Cook 29 (Table 4.30), so that all the lignin removed from the wood should be available for recovery.

The efficiency of recovery depends, in the first place, on removing all remaining liquor from the

digester and on solvent washing of the cooked chips to remove lignin deposited from the retained liquor. The problem then arises of separating the lignin from the spent liquor. In the BEC process the liquor is evaporated, first in a "flash drum" and subsequently in a "recovery tower of baffle-tray configuration" (64). The processes are apparently carried out under vacuum so that the "lignin precipitates in solid particulate form, rather than sticking and plating out on tray surfaces". However, experience in the present project did not confirm this expectation. It was found that even under vacuum, attempts to concentrate liquors in a flash evaporator cause "plating out" of liquor inside the evaporator to such an extent that the method had to be abandoned.

Instead, lignin was first precipitated by adding the liquor to 1 - 2.5 volumes of acidified water. This method succeeded in overcoming the plating out problem, and also provided the lignin in a form convenient for handling. However, the recovery was disappointingly low, and only partly accounted for by the analyses. To quote the extreme case, Cook 31, the blown liquor contained 56% of the original

lignin; 34% was recovered in the precipitate, but apparently only 2% passed into the filtrate, leaving 20% unaccounted for.

It should be noted that the foregoing figures refer to Klason lignin only, since acid soluble lignin could not be determined. It is scarcely conceivable that 20% of the Klason lignin could have been lost in the process of precipitation and filtration. A question therefore, arises about the validity of the analytical data. The Klason determination was chosen since it was the only established procedure which appeared applicable to the present system (permanganate oxidation was ruled out because of the reducing sugars present). However, it should be borne in mind that the method was developed for the analysis of wood and pulps, and its applicability to organosolv pulping liquors has yet to be confirmed. In the light of the present findings it appears that the method ought to be verified, or an alternative method developed.

5.2 Fermentation of Hemicellulose Sugars

The concentrated spent liquor from Cook 5 was passed to B.C. Research for an investigation of fermentability.

Three cultures were tested, namely Saccharomyces cerevisiae, Pachysolen tannophilus and Clostridium acetobutylicum. In the first phase of the study no microbial growth and no fermentation activity was observed with any of the cultures, with the liquor diluted to as little as 10% of the original concentration. In the second phase, the liquor was added to the synthetic control medium in varying proportions, and fermentation activity checked. All three cultures grew well in the presence of 5% of the liquors. Fermentation activity with C. acetobutylicum was suppressed by addition of 10% liquor, and activity with all three cultures was suppressed by 25% liquor.

It was concluded that toxicants were present which inhibited growth of the cultures. The toxicants were not identified, but there would appear to be two likely sources. In the first place the concentrated liquor still contained 7% of the original wood lignin, which could account for at least part of the toxicity. Secondly it is now reasonably clear that under the cooking conditions used quite extensive hemicellulose degradation can occur, leading to a variety of possibilities for the formation of toxic degradation products.

Whatever the sources, the presence of the toxicants is a formidable obstacle to the development of an organosolv pulping process which aims to produce saleable products by fermentation of hemicellulose sugars.

Copies of the two relevant B.C. Research reports are appended to this report.

5.3 Paper Properties

The plots in Figures 4.3-4.5 show that the tensile and burst strengths of the single-stage organosolv pulps passed through a maximum at about 63% yield, and tear at about 70%. An intermediate yield of 66% offers a fair compromise, giving close to maximum for all three strength parameters. Strengths at this yield were about the same as those of the sulfite reference pulp, and somewhat lower than with the kraft, as shown by the figures in Table 5.1. The limited data available from Cook 7 indicate that the organosolv pulp strength could be improved by the use of a 3-stage process, the effect being particularly marked with tear strength.

Strength comparison between pulps is complicated by the effect of pulp yield. With both kraft and sulfite, the strength decreases as the yield is increased above about

55% (80). Thus if the comparison were made at 66% yield it would undoubtedly be more favourable to the organosolv pulps. However, it seems more reasonable to base the comparison on the reference pulps at a yield corresponding to production practice, that is no more than about 55%.

In strength characteristics, the single-stage organosolv pulps can thus be considered to be, at optimum quite similar to sulfite pulps, and the question of their marketability must be closely related to the marketability of hardwood sulfite.

Printing grade papers are made predominantly from softwood fibres, which are long, giving good sheet strength, but are also often rather coarse, leading to a rather open and uneven sheet structure. Short fibered hardwood pulps are often added to fill up the gaps, giving improved opacity, smoothness and printability. The strength, which is inherently lower with hardwood pulps, is not regarded as particularly important since the filler pulps are only used to the extent of 10-20% of the total.

At present, there is a good demand for hardwood pulps. This refers essentially to hardwood kraft since relatively little hardwood sulfite is now made in North America. However, it seems most likely that if a paper grade hardwood sulfite type pulp were produced by an organosolv process it would find acceptance as a filler pulp in printing grades of paper, in spite of its somewhat lower strength.

At the same time, there would almost certainly be a problem in hemicellulose recovery. Since the organosolv pulps were, at best, somewhat weak, it would clearly be desirable to produce paper grade pulps at the highest strength attainable, which means at yields close to 66%. The plot in Figure 5.1 shows that retention of hemicellulose increases very rapidly with increasing pulp yield, and that at a yield of 66%, about half of the original wood hemicellulose is retained in the pulp and therefore unavailable for recovery. Recovery would of course be improved if lower strength pulp could be tolerated, allowing pulping to lower yields.

A similar consideration applies to the retention of lignin. The plot in Figure 5.2 shows that at the pulp yield of 66%, 30% of the original wood lignin was

retained in the pulp. Unlike hemicellulose, which is normally considered to be beneficial to pulp quality (strength), the presence of lignin is detrimental since it has to be bleached in order to make pulps of acceptable brightness for printing grades of paper. It would quite likely be considered uneconomical to bleach a pulp containing 30% of the original lignin, or at best, it would add a serious additional production cost.

5.4 Dissolving Pulp Properties

Evaluations carried out on a blend of the pulps from Cooks 10, 13, 14 and 15 showed that after bleaching, this material met laboratory test standards for viscose rayon manufacture. Final confirmation of acceptability would require pilot spinning trials and a full scale production trial, but the indications thus far are promising. For acetate production, on the other hand, the pulp did not meet the test standards, almost certainly due to the high level of residual hemicellulose. It had been expected that the quite severe alkali extraction (E stage) given to this pulp would have lowered the hemicellulose content considerably but for some reason the residual hemicellulose appeared resistant to alkaline degradation.

For an acceptable acetate grade pulp it is normally considered that the xylan and mannan levels should each be no greater than about 1.3% after bleaching. Before bleaching, a liberal estimate for the levels which could be tolerated would be about 3%. On that basis, only those cooks carried out under the severest conditions would have produced pulps which would qualify, i.e. Cooks 19, 22, 24, 25, 26, 29 and 30. The highest pulp viscosity recorded among these cooks was 2.5 cp (Cook 19), which is well below the lower limit of about 10 cp normally required in acetate grade pulps. Therefore, even though only one evaluation was carried out, it seems there might well be an insurmountable problem in attempting to produce an acceptable acetate grade dissolving pulp using a single stage methanol process.

However, it is not certain how much of that xylan would have to be removed to make an acceptable acetate pulp. Viscose grade pulps are normally required to contain no more than about 5% hemicellulose, indicated by the S18 fraction, yet the pulp tested here met the laboratory test specifications even though the S18 fraction was 12.4%. It thus appears that organosolv hemicellulose may be less troublesome in dissolving applications than the hemicellulose normally encountered in kraft and sulfite pulps.

6. CONCLUSIONS

6.1 Specific Conclusions

1. Aspen wood chips can be pulped with aqueous methanol to give organosolv pulps of varying yield depending on the severity of the cooking conditions, particularly the cooking time and acidity of the cooking liquor.
2. Under conditions of high acidity it is possible to reduce the content of hemicellulose and lignin in the pulp to less than 5% of the amounts in the original wood, using a single-stage cook of short duration, but under those conditions the pulp is so degraded as to be useless for papermaking or dissolving pulp applications.
3. Maximum pulp strength is achieved by cooking under mild conditions, to a pulp yield of about 66%; at that yield the pulp was found to be somewhat weaker than kraft but could probably still be used as a filler pulp in printing grades of paper, for which there is a good demand.
4. Pulp strength can only be achieved at the expense of loss in recoverable hemicellulose and lignin.

At 66% yield the organosolv pulp retained 50% of the original hemicellulose and 30% of the lignin, which would therefore be unavailable for any other utilization. In addition, the lignin would have to be bleached, the cost of which could well prove prohibitive.

5. A single-stage methanol cook appears capable of producing an acceptable viscose grade dissolving pulp from aspen chips, in spite of a high retention of xylan by the pulp. The process may be incapable of producing an acceptable acetate grade pulp because of the difficulty of preserving viscosity while at the same time removing deleterious hemicellulose.
6. The main obstacle to recovery of hemicellulose, for use as a raw material, is the retention by the pulp. Once extracted, there is a tendency towards degradation, particularly in cooks at high acidity, but from the data it appears that conditions could be adjusted to prevent this.
7. Toxic substances are formed, or extracted, during the cook which inhibit fermentation of the

hemicellulose sugars, except at spent liquor concentrations so low as to be commercially impractical. A solution to this problem must clearly be found if organosolv sugars are to be used as a fermentation substrate.

8. Lignin does not appear to be destroyed during the cook. At the same time, recovery of lignin after precipitation with acidified water scarcely exceeded 50% of the original. However, there is some doubt about the applicability of the Klason lignin determination to organosolv cooking liquors. For a more rigorous treatment the method needs to be checked, which may point to the necessity for developing an alternative method.
9. Methanol is not consumed in the cooking process and is theoretically available for 100% recovery. However, it is unlikely that this could be achieved economically in practice. A conservative estimate for ethanol losses likely to occur in the BEC alcohol pulping and recovery process is 3 kg/tonne of pulp produced.

6.2 Assessment

The practical realization of an alcohol pulping process which will separate wood into its three major components, each of which can be utilized as a raw material, is beset by a number of problems which have scarcely been considered in the literature.

In the single-stage methanol process there is an inherent conflict between the requirements of extracting the lignin and hemicellulose on the one hand, and producing pulp of acceptable viscosity and strength on the other. In pulping aspen chips with methanol, not much more than 50% of the original hemicellulose and 70% of the lignin could be removed without impairing pulp strength. This places a serious limitation on the availability of the raw components. In addition, the remaining lignin would have to be bleached which could well render the process uneconomic. Then there are certain serious practical problems which have to be overcome.

Firstly, a satisfactory method has yet to be devised for the separation of dissolved lignin from dissolved hemicellulose. Simple evaporation, as proposed in the BEC process, leaves the lignin in the form of a gummy

sludge which appears to all practical purposes totally intractable since it sticks to everything it comes in contact with. In the present work the lignin was precipitated with acidified water. This was only partially successful: thus, even though the concentration of acid soluble lignin in the filtrate could not be determined it was evident that a fair amount of lignin remained in solution, from the amount deposited in the flash evaporator on subsequent evaporation. Furthermore, the addition of up to 2.5 volumes of water adds tremendously to the amount of evaporation needed to concentrate the hemicellulose.

Secondly, there is a very serious problem in the toxicity developed in the spent cooking liquor. After condensation the raw liquor is to all intents, unfermentable and clearly needs treatment for the removal of the toxicants. Since the toxicants have not as yet been identified it is impossible even to guess at the magnitude of the task likely to be involved.

The somewhat scant information which could be made available for multi-stage cooking makes it difficult to give an assessment of that process relative to the single-stage. In the BEC three stage process the chips

are cooked for relatively short periods of time and in the light of the present findings that appears a highly desirable feature. On the other hand, the problems of lignin-hemicellulose separation and toxicity would doubtless remain to be solved. For a full assessment of the process obviously more experimental work is required.

7. RECOMMENDATIONS

1. The single-stage methanol process is beset by a number of serious problems, at least one of which appears inherent in the process and not amenable to any solution. It is therefore recommended that no further work should be carried out on single-stage cooking.
2. The BEC 3-stage process appears to offer a significant advantage in that the cooking time is kept short, which should minimize hemicellulose and cellulose degradation. Questions remaining unanswered concern how much lignin and hemicellulose can be extracted in the various stages, and how these can be separated efficiently. However, perhaps the most important question concerns the toxicants which are present in the spent cooking liquors. Insofar as the viability of the process depends on being able to ferment the hemicellulose sugars, the first priority should be to find ways of removing or deactivating the toxicants.
3. An alternative which does not so far seem to have been considered, concerns the possibility of avoiding the use of an organic solvent in the first stage and using water instead. There is evidence that hemicellulose is extracted more readily from wood with water than with

alcohol (25). Pretreatment with water or dilute acid is certainly well known in kraft pulping as a means of removing unwanted hemicellulose (prehydrolysis). At the same time, wood lignin is substantially insoluble in water. Thus a preliminary pulping stage in which wood chips are heated with water should provide an effective route to removing sugars with minimum contamination by lignin. The lignin would then be extracted in a second stage, using an alcohol water mixture. In this way the hemicellulose and lignin should be obtained already substantially separated from each other, avoiding one of the major problems with the existing processes. It is recommended that experimental work should be undertaken to investigate the potential of a two-stage water/alcohol cooking process.

8. REFERENCES

1. Kleinert, T., and Tayenthal, K. *Angew. Chem.*, 44, 788, (1931); U.S. Patent 1,856,567 (1932).
2. Hartmuth, R., German Patents 326,705; 328,783 (1919).
3. Aronovsky, S.I. and Gortner, R.A., *Ind. Eng. Chem.*, 28, 1270 (1936).
4. Grondal, B.L. and Zenczak, P., U.S. Patent 2,772,968 (1956).
5. Engel, O. and Wedekind, E., German Patent 581,806 (1933).
6. Bailey, A.J. et al., *Ind. Eng. Chem.* 30, 1407 (1938); *Pacific Pulp Pap. Ind.*, 14, No. 3, 24 (1940).
7. Hibbert, H. and Rowley, H.J., *Can. J. Res.*, 2, 357 (1930).
8. Machell, G. and Richards, G.N., *J. Chem. Soc.*, 1944 (1960).
9. Furman, K.E. and Walt, P.C., U.S. Patent 2,760,961 (1956).
10. Rassow, B., and Gabriel, H., *Cellulosechem.*, 12, 227, 249, 290, 318 (1931).
11. Aronovsky, S.I., and Gortner, R.A., *Ind. Eng. Chem.*, 29, 1431 (1937).
12. Katzen, R., Pearlstein, J., Muller, R.E. and Othmer, D.F., *Tappi*, 33, 67 (1950).
13. Giertz, H.W. and McPherson, J., *Norsk. Skogind.*, 10, 348 (1956).
14. Schorning, P., *Faserforsch. Textiltech.*, 8, 487 (1957).
15. Springer, E.L., Harris, J.F. and Neill, W.K., *Tappi*, 46, 551 (1963).
16. Kleinert, T.N., U.S. Patent 3,585,104 (1968).
17. Kleinert, T.N., *Oesterr. Papier*, 11, No. 11, 4, 16, 18 (1974).

18. Kleinert, T.N., Tappi, 57, No. 8, 99 (1974).
19. Kleinert, T.N., Tappi, 58, No. 8, 170 (1975).
20. Kleinert, T.N., Holzforschung, 29, No. 3, 107 (1975).
21. Kleinert, T.N., Papier, 30, No. 10A, V18 (1976).
22. Bowers, G.H., and April, G.C., Tappi, 60, No. 8, 102 (1977).
23. April, G.C., Kamal, M.M., Reddy, J.A., Bowers, G.H. and Hansen, S.M., Tappi, 62, No. 5, 83 (1979).
24. April, G.C., Bharoocha, R., Sheng, J. and Hansen, S., Tappi, 65, No. 2, 41 (1982).
25. McGee, J.K. and April, G.C., Chem. Eng. Commun., 19, 49 (1982).
26. Hansen, S.M. and April, G.C., Ind. Eng. Chem. Prod. Res. Div., 21, 621 (1982).
27. Baumeister, M., and Grobenzell, E., German Patent 2,855,052 (1980).
28. Meshitsuka, G., Japan Tappi, 34, No. 3, 263 (1980).
29. Daima, H., Hosoya, S. and Nakano, J., J. Japan Wood Res. Soc., 22, No. 9, 539 (1976).
30. Nakano, J., Takatsuka, C. and Daima, H., Japan Tappi, 30, No. 12, 650 (1976).
31. Nakano, J., Takatsuka, C., Daima, H., Kobayashi, M. and Sasaki, N., Japan Tappi, 31, No. 11, 762 (1977).
32. Daima, H., Hosoya, S. and Nakano, J., Japan Tappi, 32, No. 4, 245 (1978).
33. Daima, H., Hosoya, S., Nakano, J. and Ishizu, A., Japan Tappi, 33, No. 6, 418 (1979).
34. Nakano, J., Daima, H., Hosoya, S. and Ishizu, A., SPCI Internat. Symp. Wood and Pulping Chem., (Stockholm) Preprints, 2, 72 (1981).
35. Kobayashi, M., Sasaki, N., Gobara, K. and Nagasawa, T., Japan Tappi, 32, No. 9, 525 (1978).

36. Marton, R. and Granzow, S., Tappi, 65, No. 6, 103 (1982).
37. McKee, J.K. and April, G.C., Proc, Pacific Chem. Eng. Congr. (Seoul, Korea) 3d, 4, 209 (1983).
38. Green, J. and Sanyer, N., Tappi, 65, No. 5, 133 (1982).
39. Baumeister, M. and Edel, E., German Patent 3,212,767 (1983).
40. Wang, D.I.C. and Avgerinos, C., U.S. Patent 4,395,543 (1983).
41. Rayonier Inc., British Patent 1,217,573 (1970).
42. Primakov, S.F., Tsarenko, I.M. and Zaplatin, V.P., Khim. Drev. (Riga) No. 6, 39 (1979).
43. Sommer, K. and Spatz, W., German Patent 2,838,380 (1980).
44. Bublitz, W.J., Hull, J.L., Wilson, K. and Gidlof, P., Int. Symp. Wood & Pulping Chem. (Japan) 3, 38 (1983).
45. Bublitz, W.J. and Hull, J.L., Proc. Tappi Int. Sulfite Pulping Conf. (Toronto) 263 (1982).
46. Bublitz, W.J. and Wilson, K.P., Proc. Tappi Pulping Conf. (Houston) 2, 423 (1983).
47. Primakov, S.F., Tsarenko, I.M. and Antonenko, L.P., Khim. Drev. (Riga) No. 4, 23 (1982).
48. Sakai, K., Mun, S.P. and Imura, H., J. Japan Wood Res. Soc., 29, No. 3, 291 (1983).
49. Bublitz, W.J., Preprint, Am. Inst. Chem. Eng., Summer Meeting, Seattle (1985).
50. Rickey, R.G. and Lovell, E.L., Canadian Patent 871,041 (1971).
51. Sarkanen, K.V., U.S. Patent 4,329,200 (1982).
52. Nimz, H.H. and Casten, R., Proc. Int. Symp. Wood and Pulping Chem., (Vancouver) 265 (1985).

53. April, G.C., Aravamuthan, R., Chiang, V., Zargarian, K., Chen, W.Y, Westmoreland, R. and Meraab, J., Preprint, Am. Inst. Chem. Eng., Summer Meeting, Seattle (1985).
54. Haggin, J., Chem & Eng. News, 64, No. 4, 33 (1986).
55. Anon., Pulp & Paper, 60, No. 2, 33 (1986).
56. Sarkanen, K.V., Prog. Biomass Conversion, 2, 127 (1980).
57. Paszner, L. and Chang, P-C., Proc. Int. Symp. Wood Pulping Chem. (Tsukuba, Japan) supplement volume, 21 (1983).
58. Paszner, L., Proc. Tappi Int. Dissolving & Specialty Pulps Conf., 81 (1983).
59. Antal, L., Paszner, L., Bereha, N. and Micko, M.M., Proc. Tappi Res. & Dev. Conf. (Atlanta) 223 (1984)
60. Paszner, L. and Behera, N.C., Holzforschung, 39, 51 (1985).
61. Paszner, L. and Chang, P-C., Canadian Patent 1,100,266 (1981).
62. Chang, P-C. and Paszner, L., Canadian Patent 1,131,415 (1982).
63. Chang, P-C., and Paszner, L., Canadian Patent 1,150,012.
64. Katzen, R., Chem. Eng. Prog., No. 2, 62 (Feb. 1980).
65. Lora, J.H. and Aziz, S., Tappi J., 68, No. 8, 94 (1985).
66. Lora, J.H., Cronlund, M., Powers, J., Orlowski, G. and Wu, L., Proc. Int. Symp. Wood & Pulping Chem. (Vancouver) 181 (1985).
67. Diebold, V.B., Cowan, W.F. and Walsh, J.K., Canadian Patent 1,079,008 (1980); U.S. Patent 4,100,016 (1978).
68. Anon., Papier Druck, 90, No. 9, 9, 11 (1984).
69. Edel, E., Deut. Papierwirt., No.1, 39 (1984).

70. Lipinsky, E.S., Tappi J., 66, No. 10, 47 (1983).
71. Gomide, J.L., Ph.D. Thesis, N. Carolina State U., Raleigh, NC, 1978, p. 13.
72. Ayla, C., Holzforschung, 36, No. 2, 93 (1982).
73. Griffith, W.L., Compere, A.L., Rao, T.K. and Griest, W.H., Proc. Tappi Papermakers Conf. (Atlanta) 181 (1980).
74. Anon., Pulp & Paper Canada, 85, No. 7, 15 (1984).
75. Pye, E.K., Pulp & Paper Canada, 85, No. 12, 8 (1984).
76. Forgacs, O.L., Pulp & Paper Canada, 85, No. 10, 7 (1984).
77. Pigman, W.W. and Goepp, R.M., "Chemistry of the Carbohydrates", Academic Press, New York, 1948, p. 71.
78. Sarkanen, K.V., Discussion at U. of Washington, July 3, 1985.
79. Post's Pulp & Paper Directory, 1986, p. 525.
80. Rydholm, S.A., "Pulping Processes", Interscience Publishers division of John Wiley and Sons, Inc., New York-London-Sydney, 1965, pp. 546, 645.

9.1 Appendix A

TABLES OF RESULTS

Table 3.1

Analysis of Trembling aspen chips

	<u>Batch 1</u>	<u>Batch 2</u>	<u>Batch 3</u>
When obtained	Fall 1984	Oct 1985	Jan 1986
Used in cooks	2-16	17-26	29-31
Moisture, %	47.7	40.5	53.6
Arabinan, %	0.6	0.5	0.5
Xylan, %	19.6	18.6	17.9
Mannan, %	2.3	1.2	1.9
Galactan, %	1.0	0.5	0.6
Glucan, %	45.3	44.4	46.1
Klason lignin, %	19.9	20.3	20.2
Total lignin, %	23.4	23.2	23.3
DCM extractives, %	2.21	1.43	1.34

Table 3.2

Evaporation of 80:20 methanol-water under vacuum between 500 and 580 mm Hg

Sample	Cumulative evaporation % w/w	MeOH left % v/v	Temperature deg C
0	0	82	33
1	47.3	62	43
2	69.4	48	55
3	83.4	21	57
4	92.7	< 0.5	67
5	96.7	-	70
6	98.5	-	70

Table 4.1

Preliminary single-stage ethanol cooks

Aspen chips (3 kg o.d.) cooked with 80:20 ethanol-water,
0.05 molar in CaCl_2 and 0.0015N in HCl ,
liquor to wood ratio 9.3:1

	-----Cook No.-----						
	2	3	4	5	8	Kraft	Sulfite
Time to 190 deg C, min	53	48	49	60	54		
Time at 190 deg C, min	60	20	40	20	60		
Yield, %	56.2	61.4	53.8	55.9	52.8	56.5	54.0
Kappa Number:							
Water washed	44.8	53.9	48.2	-	-	11.5	10.1
Alcohol washed*	13.1	25.0	15.8	16.0	10.7		
Acetone washed**	12.4	22.9	18.3	20.1	-		
Viscosity, 0.5% CED, cp:							
Water washed	13.9	24.4	12.1	-	-	61.8	24.4
Alcohol washed*	14.0	28.6	12.1	16.3	8.4		
Acetone washed**	14.3	29.5	12.3	16.9	-		

* 17 l 80:20 ethanol at 50 deg C, after screening and water washing, followed by 50 deg C water and finally cold water.

** 10 l at room temperature, after screening and water washing, followed by 50 deg C and cold water.

Table 4.2

Physical properties of single stage ethanol pulps

	CSF ml	PFI revs	Burst Index kPa.m2/g	Tear Index mN.m2/g	Tensile BL, km	Bulk cm3/g
Cook No. 2	512	0	2.6	5.5	7.3	1.46
	500	100	2.7	5.5	7.4	1.44
	400	1030	3.5	5.5	8.2	1.34
	300	2520	4.1	5.2	8.5	1.29
Cook No. 3	506	0	2.5	5.2	6.7	1.53
	500	30	2.5	5.2	6.9	1.52
	400	560	3.4	5.4	8.6	1.42
	300	1510	4.1	5.3	9.1	1.35
Cook No. 4	519	0	2.0	4.1	6.1	1.54
	500	80	2.1	4.1	6.2	1.52
	400	710	2.6	4.5	6.6	1.41
	300	1830	2.9	4.6	7.2	1.35
Cook No. 5	508	0	2.1	4.8	6.4	1.49
	500	30	2.2	4.8	6.4	1.48
	400	490	2.8	4.8	7.4	1.38
	300	1590	3.0	4.8	7.8	1.33
Cook No. 8	554	0	1.9	4.0	4.9	1.53
	500	200	2.3	4.0	5.6	1.44
	400	860	2.9	4.0	6.8	1.35
	300	2340	3.3	4.0	7.2	1.30
Kraft	562	0	2.8	7.6	7.6	1.48
	500	300	3.7	7.6	8.7	1.44
	400	1400	4.9	7.7	9.8	1.38
	300	3600	5.7	8.0	10.2	1.35
Sulfite	571	0	2.9	6.9	7.0	1.43
	500	600	3.4	7.7	7.8	1.42
	400	1800	4.1	7.1	8.3	1.36
	300	4300	4.7	6.0	8.6	1.26

Table 4.3

Caustic extraction of alcohol washed pulp from Cook 3

NaOH Conc, %	Temp deg C	Time minutes	Yield %	Kappa No.	Viscosity 0.5%, cp	S18 %
-	-	-	(100)	25.0	28.6	14.8
0.1	Room	10	-	17.5	28.4	-
0.1	Room	30	-	19.2	27.2	-
0.1	Room	60	-	18.6	27.5	-
0.5	Room	30	96.6	14.5	30.9	13.9
0.5	30	30	96.5	15.1	26.9	13.9
0.5	75	60	95.2	14.8	28.3	13.1

Table 4.4

Physical properties of pulps after extraction with NaOH

	CSF ml	PFI revs	Burst Index kPa.m ² /g	Tear Index mN.m ² /g	Tensile BL, km	Bulk cm ³ /g
Cook No. 3						
Extracted 0.5% NaOH soln						
1. 30 min, room temp	523	0	2.5	5.4	7.0	1.45
	500	100	2.7	5.4	7.3	1.43
	400	800	3.5	5.5	8.3	1.34
	300	2400	4.1	5.5	8.8	1.29
2. 30 min, 60 deg C	528	0	2.4	5.4	5.9	1.48
	500	100	2.5	5.5	6.3	1.45
	400	700	3.1	5.7	7.4	1.36
	300	2650	3.9	5.8	8.5	1.31
3. 60 min, 75 deg C	538	0	2.0	5.3	5.2	1.48
	500	100	2.4	5.4	5.8	1.45
	400	900	3.0	5.7	7.3	1.36
	300	2600	3.7	6.0	8.1	1.30
Cook No. 3						
Unextracted						
	506	0	2.5	5.2	6.7	1.53
	500	30	2.5	5.2	6.9	1.52
	400	560	3.4	5.4	8.6	1.42
	300	1510	4.1	5.3	9.1	1.35
Cook No. 8						
Extracted 0.1% NaOH soln						
30 min, room temp	556	0	1.9	4.1	4.6	1.50
	500	230	2.2	4.2	5.2	1.44
	400	1040	2.6	4.3	6.1	1.36
	300	2300	3.1	4.6	6.8	1.31
Cook No. 8						
Unextracted						
	554	0	1.9	4.0	4.9	1.53
	500	200	2.3	4.0	5.6	1.44
	400	860	2.9	4.0	6.8	1.35
	300	2340	3.3	4.0	7.2	1.30

Table 4.5

Material balance for Cook 5

	Pulp grams	Isolated Lignin grams	Liquor grams	Total grams	Original Wood grams	Analytical Recovery %
Arabinan	3.3	0.4	4.6	8.3	18	46
Xylan	164	6.2	113	283	588	48
Mannan	47	0.4	5.5	53	69	77
Galactan	13.4	-	3.9	17.3	30	58
Glucan	1276	0.7	6.2	1283	1359	94
Total Hemi	228	7.0	127	362	705	51
Total Carbohydrate	1504	7.7	133	1645	2064	80
Soluble lignin	8.4	29	23	60	105	57
Klason lignin	103	291	24	418	597	70
Total lignin	111	320	47	478	702	68
A/B extracts	-	-	-	-	66	-
Total solids found by analysis	1616	328	180	2123	2832	
Total solids in starting material	1677	367	446	2490	3000	
% accounted for by analysis	96	89	40	85	94	

Table 4.6

Cooking conditions for higher yield cooks
with 80:20 methanol-water

	Cook Number						
	10	11	12	13	14	15	16
Weight of chips, g o.d.	365	365	365	400	400	400	400
Molarity of MgSO ₄	0.024	0.024	0.024	0.05	0.05	0.05	0.05
Liquor to wood ratio	22:1	22:1	22:1	20:1	20:1	20:1	20:1
Minimum temp after cook start, deg C	171	182	183	173	180	171	174
Time minimum temp read, min	2	3	2	2	5	3	5
Time to regain 190 deg C, min	16	12	9	17	23	60	60*
Total cooking time, hr	2	1	0.5	4	3	6	2
pH at 5 min	-	4.8	4.9	-	4.8	4.9	4.9
pH at 60 min	4.6	4.7	-	4.5	4.6	4.7	4.7
pH at end of cook	4.6	4.7	4.7	4.6	4.6	4.6	4.7

[*186 deg C - electric heater failed]

Table 4.7

Pulp analyses for the higher yield single-stage methanol cooks

	Cooking Time, hours						
	0.5	1	2	2	3	4	6
Cook number	12	11	10	16	14	13	15
Yield, %	76.4	68.8	57.8	68.4	63.3	59.7	59.2
Viscosity, 0.5% CED, cp	53.7*	75.8*	41.7	60.6	47.8	38.6	34.4
Kappa No.	68.9	61.9	25.2	51.1	30.4	20.3	21.0
Klason lignin, %	12.7	10.8	6.5				
Soluble lignin, %	3.7	1.8	1.3				
Carbohydrates, %							
Xylan	16.4	15.2	11.9	15.0	14.3	12.8	13.7
Mannan	2.0	2.0	2.2	2.1	2.2	2.1	2.0
Galactan	0.3	0	0	0.1	0	0	0
Glucan	58.9	66.4	75.2	62.4	63.9	65.5	66.4
S18	15.2	14.9	14.1	18.1	17.4	15.9	15.0
S10	20.2	20.3	16.5	20.6	19.2	17.2	16.9

* partially insoluble

Table 4.8

Physical properties of the higher yield single-stage methanol pulps

	CSF ml	PFI revs	Burst Index kPa.m2/g	Tear Index mN.m2/g	Tensile BL, km	Bulk cm3/g
Cook No. 10	522	0	2.4	6.2	6.5	1.58
	500	200	2.6	6.2	7.0	1.53
	400	1050	3.6	6.3	8.5	1.41
	300	2500	4.2	6.3	9.2	1.35
Cook No. 11	524	0	2.1	6.4	6.0	1.69
	500	200	2.4	6.5	6.4	1.65
	400	1800	3.3	6.3	7.8	1.53
	300	4300	4.1	6.1	8.7	1.44
Cook No. 12	714	0	0.7	4.9	3.1	2.01
	600	2250	2.0	5.9	5.5	1.71
	500	5400	2.6	6.0	6.5	1.62
	400	9600	2.9	5.9	7.0	1.55
	300	14400	3.3	5.8	7.3	1.50
Cook No. 13	451	0	3.1	5.6	8.0	1.40
	400	540	3.7	5.5	8.4	1.38
	300	2280	4.1	5.3	9.3	1.27
Cook No. 14	485	0	2.8	5.8	7.0	1.52
	400	740	4.1	5.7	9.1	1.44
	300	2980	4.6	5.6	9.6	1.36
Cook No. 15	453	0	3.3	5.8	8.0	1.45
	400	120	3.6	5.7	8.4	1.44
	300	2280	4.4	5.4	9.5	1.34
Cook No. 16	428	0	3.2	6.2	8.0	1.52
	400	200	3.5	6.1	8.2	1.51
	300	1720	4.4	6.0	9.0	1.44

Table 4.9

Bleaching conditions for dissolving grade pulps

Stage	Designation	Chemical % on pulp	Time min	Temperature deg C	Consistency* %
<u>Viscose</u>					
1	C	5% Cl ₂	45	30	3.5
2	E	10% NaOH	30	128	10
3	H	0.9% NaOCl	120	60	9
4	H	0.6% NaOCl	240	60	9
5	D	0.25% ClO ₂	225	65	9
<u>Acetate</u>					
1	C	5% Cl ₂	45	30	3.5
2	E	18% NaOH	35	145	10
3	H	0.7% NaOCl	105	60	9
4	D	0.25% ClO ₂	210	65	9

* % pulp in the mix of pulp and liquor

K No: starting material - 16
after E stage: viscose - 1.0, acetate - 0.9

Table 4.10

Analysis of dissolving grade pulps

	Viscose	Acetate
S18, %	12.4	11.0
S10, %	-	13.8
Xylan, %	-	11.3
Mannan, %	-	1.2
Dichloromethane extractives, %	0.025	0.049
Viscosity, 0.5% CED, cp	29	72

Table 4.11

Evaluation of bleached organosolv pulp blend
for acetate rayon production

	Organosolv Pulp	Commercial Acetate Pulps
Reaction time to target dope viscosity, min	154	60-90
<u>Secondary acetate properties</u>		
Haze, 10^{-3} cm^{-1}	520	20-50
Colour (coefficient of yellowness)	0.6	0.25-0.4
Filterability:		
Plugging Value, g/cm ²	8	25-55
(Clogging Constant) ⁻¹ , ml	3	8-60

Table 4.12

Evaluation of bleached organosolv pulp blend
for viscose rayon production

	Organosolv Pulp	Commercial Viscose Pulps
Ageing time (cellulose depolymerisation to target viscosity), hours	4.1	2-8
Reaction time to target CS2 absorption, min	43	37-47
Clogging constant, 10^{-3} ml^{-1} (inverse filterability)	21	12-50
Clarity, cm	3.7	2-22
Haze, 10^{-3} cm^{-1}	185	50-300
Particle count, 10^{-6} m : +19	475	200-1200
+40	5	4-30

Table 4.13

Analysis of liquors and lignin from
higher yield single-stage methanol cooks

	Liquor Vol, l	-----Liquor Composition, mg/l-----					Klason Lignin
		Xylan	Mannan	Galactan	Glucan		
<u>Combined liquors, Cooks 10-12</u>							
Defibering & washing	34.7	123	5	13	5	660	
Blow liquor	14.7	2330	99	107	80	-	
Evaporated liquor	27.0	1330	58	53	66	440	
<u>Combined liquor, Cooks 13-16</u>							
Evaporated liquor	21.0	1940	71	90	68	860	
	Air dry wt, g	-----Lignin Composition, % w/w-----					Klason Lignin
		Xylan	Mannan	Galactan	Glucan		
<u>Precipitated lignin</u>							
Cooks 10-12	85	1.7	0.1	0	0.5	91.6	
Cooks 13-16	119	0.9	0.1	0	0.2	90.1	

Table 4.14

Recovery of carbohydrates from higher yield single-stage methanol cooks

	-----% of component recovered-----				
	Xylan	Mannan	Galactan	Total Hemi	Glucan
<u>Combined Cooks 10-12</u>					
Defibering & wash liquor	2.0	0.7	1.6	2.0	0
Evaporated liquor	16.7	6.2	13.0	15.5	0.4
Pulp	50.8	60.5	7.6	48.6	98.7
Lignin	0.7	0.3	0	0.6	0.1
Total	70.2	67.7	22.2	66.7	99.2
<u>Combined Cooks 13-16</u>					
Evaporated liquor	13.0	4.1	11.8	12.0	0.3
Pulp	44.8	57.3	1.7	43.0	89.2
Lignin	0.3	0.3	0	0.3	0
Total	58.1	61.7	13.5	55.3	89.5

Table 4.15

Aspen chips (3 kg o.d.) cooked with 80:20 methanol-water
 0.01 molar in H₂SO₄, liquor to wood ratio 9.3:1
 Time to reach 190 deg C: 48-50 min

Time at 190 deg C, min	Variations in pH									
	-----					Cook Number -----				
	17	18	19	20	21	22	23	24	25	26
Starting liquor	2.1	1.9	1.9	1.9	2.0	2.0	1.9	1.9	1.9	1.9
5	3.7	3.1	2.9	2.8	3.0	2.9	3.0	2.8	2.7	2.8
30	3.8	3.4	3.1	3.0	3.3	3.0	-	2.9	2.9	2.9
60	4.0	3.5	3.3	3.2	3.5	3.1	3.1	3.0	2.9	3.0
90	4.1	3.8	3.4		3.6			3.0	3.0	
120	4.2	3.9	3.6		3.7			3.1	3.0	
150		3.9	3.6					3.1	3.1	
180		3.9	3.7					3.2	3.1	
210			3.7							
240			3.8							

Table 4.16

Properties of pulps produced in the cook series to generate hemicellulose

	Cook Number								
	17	18	19	20	21	22	24	25	26
Cooking time, h	2	3	4	1	2	1	3	2.73	1
pH at half time	4.0	3.8	3.6	3.0	3.5	3.0	3.0	3.0	2.9
Pulp yield, %	59.3	51.3	46.2	48.2	49.8	45.7	38.1	37.9	44.7
Viscosity, 0.5% CED, cp	14.8*	4.3	2.5	3.0*					
Kappa No.	48.5	43.9	42.1	48.9					
Klason lignin, %	6.78	3.63	3.68	6.89	4.16	3.19	3.20	3.40	1.58
Soluble lignin, %	1.56	6.44	0.75	0.46					
Carbohydrates, %									
Xylan	11.6	5.8	2.7	4.0	4.7	2.0	1.8	0.6	2.2
Mannan	1.3	0.7	0.4	0.5	0.5	0.3	0.3	0.2	0
Glucan	76.6	86.7	91.5	87.8	92.3	90.7	91.2	91.3	94.4
S18, %	16.2	17.0	11.2	11.6					
S10, %	24.0	34.0	33.6	37.4					

*partially insoluble

Table 4.17

Analysis of liquors in the cook series to generate hemicellulose

Cook	Sample	----- Weight of component, g -----					Total Solids	MeOH % w/w
		Liquor vol, l	Xylan	Mannan	Galactan	Glucan		
	Starting (chips)		558	36	15	1332	3000	
17	30 min	27.8	82	3	9	4	890	75.0
	63 min	27.5	78	10	10	3	1030	75.6
	90 min	27.2	180	5	9	6	1020	75.5
	120 min	26.9	54	2	4	3	1120	75.3
	Blow composite	12.5	86	3	5	3	720	
	Evap'd liquor	1	10	1	1	1		
18	5 min	28.0	130	5	11	8	-	-
	30 min	27.8	130	5	8	10	1250	76.2
	60 min	27.5	140	6	9	12	1300	76.5
	90 min	27.2	140	6	8	13	1280	76.4
	120 min	26.9	120	3	8	13	1290	76.5
	150 min	26.3	110	6	7	14	1200	75.8
	180 min	26.0	130	7	7	14	1200	77.6
	Evap'd liquor	1	31	2	1	1		
19	30 min	27.8	150	6	11	18	1420	76.6
	60 min	27.5	140	7	9	24	1380	76.6
	90 min	27.2	140	7	8	28	1590	76.2
	120 min	26.9	140	7	4	29	1550	76.4
	150 min	26.6	140	7	8	32	1540	76.7
	180 min	26.3	120	7	6	31	1500	76.6
	210 min	26.0	120	7	6	32	1420	76.3
	240 min	25.7	110	6	5	31	1500	76.3
	Evap'd liquor	1	38	2	10	5		
20	60 min	27.5	180	8	10	31	1540	76.6
	Evap'd liquor	1	51	2	5	3		

Table 4.17 continued

Cook	Sample	----- Weight of component, g -----					Total Solids	MeOH % w/w
		Liquor vol, l	Xylan	Mannan	Galactan	Glucan		
21	30 min	27.8	140	8	11	14	1420	76.3
	60 min	27.5	140	6	10	17	1470	76.6
	90 min	27.2	170	8	10	21	1490	76.5
	120 min	26.9	150	8	10	21	1460	76.1
	Evap'd liquor	1	112	5	5	13		
22	60 min	27.5	210	10	10	46	1570	76.1
	Evap'd liquor	1	140	6	6	27		
24	5 min	28.0	64	4	4	56	1280	76.6
	30 min	27.8	170	7	12	30	1520	76.8
	60 min	27.5	220	10	11	58	1650	76.3
	90 min	27.2	160	9	10	61	1680	75.9
	120 min	26.9	180	9	9	78	1700	76.1
	150 min	26.6	160	8	7	87	1730	76.4
	180 min	26.3	170	10	9	110	1710	76.6
	Evap'd liquor	1	84	5	4	44		
25	30 min	27.8	190	9	13	38	1500	76.6
	60 min	27.5	170	10	11	56	1610	76.9
	90 min	27.2	140	8	9	62	1650	76.3
	120 min	26.9	130	8	8	75	1720	75.9
	150 min	26.6	130	9	8	88	1720	75.7
	164 min	26.3	120	11	8	88	1720	76.1
	Evap'd liquor	1	78	4	4	45		
26	60 min	27.5	150	7	9	43	1620	76.3
	Evap'd liquor	1	100	5	6	29		

Table 4.18

Recovery and loss of hemicellulose, Cooks 17-26

Cook	----- Hemicellulose, % of original in wood -----						
	Liquor at cook end	Evap'd Liquor	Pulp	Sampling	Pulp + liquor + samples	Loss in cooking	Loss in processing & evaporation
	(A)	(B)	(C)	(D)	(1)	(2)	(3)
17	10	2	37	1	40	52	8
18	24	6	18	2	26	56	18
19	20	9	7	2	18	71	11
20	33	10	10	1	21	56	23
21	28	22	14	1	37	57	6
22	38	25	5	1	31	56	13
24	31	15	4	2	21	63	16
25	23	14	1	2	17	74	9
26	27	18	5	1	24	67	9

(1) Total recovered or removed, B+C+D

(2) 100 - (A+C+D)

(3) A-B

Table 4.19

Material balance, total solids, Cooks 17-26

Cook No.	----- Total solids, % of 3 kg chips -----			
	In liquor at cook end	In samples	Pulp yield	Total solids accounted for
17	37.1	1.6	59.3	98.0
18	41.8	3.0	51.3	96.1
19	50.2	4.7	46.2	101.1
20	51.0	1.3	48.2	100.5
21	49.0	2.3	49.8	101.1
22	51.9	1.3	45.7	98.9
24	56.6	3.7	38.1	98.4
25	56.6	3.9	37.9	98.4
26	53.7	1.4	44.7	99.8

Table 4.20

Material balance, lignin, Cooks 17-26

	Cook Number/Sample Time	
	<u>19/240</u>	<u>25/120</u>
% of original lignin in the cooking liquor	87	89
% of original lignin in in the pulp	8	6
Total lignin accounted for	95	95

Table 4.21

Recovery of lignin, Cooks 17-26

----- Lignin, % of original -----

Cook No.	Precipitated lignin	Lignin in pulp	Total
17	36	20	56
18	45	9	54
19	40	8	48
20	53	9	62
21	46	10	56
22	49	7	56
24	46	6	52
25	49	6	55
26	52	3	55

Table 4.22

Short duration single-stage methanol cooks

Aspen chips (3 kg o.d.) cooked with 80:20 methanol-water
liquor to wood ratio 9.3:1

	<u>Cook 29</u>		<u>Cook 30</u>		<u>Cook 31</u>	
Molarity H ₂ SO ₄	0.05		0.02		0.01	
Time to reach 190 deg C, min	42		43		45	
Time from start-up, min	Temp deg C	pH	Temp deg C	pH	Temp deg C	pH
0						
Liquor alone	20	1.2	20	1.6	20	1.8
Liquor + chips	20	1.4	20	1.9	20	-
30	179	1.7	178	2.1	170	2.9
45	190	1.9	190	2.5	190	3.3
60	190	2.0	190	2.4	190	3.3
75					190	3.6

Table 4.23

Analysis of pulps produced in short duration methanol cooks

	Cook 29	Cook 30	Cook 31
Chip yield, %	-	57.3	65.6
Pulp yield, %	-	40.7	55.2
Viscosity, 0.5% CED, cp	-	1.9	4.6
Kappa No.	-	28.3	40.8
Klason lignin, %	2.5	2.7	4.8
Soluble lignin, %	1.0	0.5	0.5
Carbohydrates, %			
Xylan	0	1.7	8.5
Mannan	0.3	0.5	1.3
Galactan	0	0	0.1
Glucan	74.2	94.8	83.8

Table 4.24

Analysis of lignins produced in short duration methanol cooks

	Cook 29	Cook 30	Cook 31
Total weight, g	425	332	275
Klason lignin, %	87.5	84.9	85.4
Soluble lignin, %	3.6	5.3	1.8
Carbohydrates, %			
Xylan	0	1.5	1.1
Mannan	0	0.3	0.2
Galactan	0	0	0
Glucan	1.2	1.8	0.4

Table 4.25

Analysis of liquors in short duration cooks

Sample	----- Weight of component, grams -----						Total Solids
	Liquor vol, l	Xylan	Mannan	Galactan	Glucan	Klason lignin	
<u>Cook 29, 0.05 m H2SO4</u>							
Cook, 30 min	28.0	380	34	16	54	450	1290
45 min	27.6	160	36	24	360	550	1880
60 min	27.2	58	35	7	460	660	1960
Blown liquor	14.75	43	28	5	350	480	1420
Filtered liquor	42.0	37	20	4	230	79	910
After 1st evap'n	15.3	31	22	-	240	-	-
After 2nd evap'n	5.91	35	23	8	290	-	-
After 3rd evap'm	3.08	37	24	5	350	-	-
Final liquor	1.24	29	22	8	298	-	-
<u>Cook 30, 0.02 m H2SO4</u>							
Cook, 30 min	28.0	230	14	10	16	290	900
45 min	27.6	320	31	14	62	620	1340
60 min	27.2	280	41	12	180	630	1520
Blown liquor	14.37	190	28	9	130	400	1050
Filtered liquor	40.22	160	26	6	120	33	650
After 1st evap'n	15.08	160	26	7	120	-	-
After 2nd evap'n	6.22	150	26	7	130	-	-
After 3rd evap'n	4.00	150	25	7	120	-	-
Final liquor	1.12	40	21	5	120	14	610
<u>Cook 31, 0.01 m H2SO4</u>							
Cook, 30 min	28.0	140	7	7	9	210	600
45 min	27.6	280	23	12	23	440	1110
60 min	27.2	330	30	14	39	510	1240
75 min	26.8	370	32	14	43	590	1370
Blown liquor	14.12	230	22	8	34	390	910
Filtered liquor	26.65	240	22	9	33	16	600
After 1st evap'n	10.80	230	19	8	30	-	-
After 2nd evap'n	4.82	210	18	7	26	-	-
Final liquor	1.12	230	20	8	28	5	500

Table 4.26

Total of hemicellulose, glucan and Klason lignin
as a percentage of the measured total solids

	Cook 29	Cook 30	Cook 31
30 min	72	62	62
45 min	60	78	70
60 min	62	75	74
75 min	-	-	77
Blown liquor	64	72	77
Filtered liquor	41	53	75
Final liquor	-	49	58

Table 4.27

Correction of pulp yield to maintain total solids balance

	Cook 29	Cook 30	Cook 31
Pulp Yield, %			
Determined	-	40.7	55.2
Calculated	32.2	47.5	(55.2)

Table 4.28

Calculation of liquor retained by chips
at the end of the cook

	Cook 30	Cook 31
Chip yield, %	57.3	65.6
Pulp yield, %	47.5	55.2
Retained solids, %	9.8	10.4
Retained solids, g	294	312
Retained liquor, l	5.26	6.12
Ratio - liquor:pulp, l/kg	3.7	3.7

Liquor retained by pulp in Cook 29, assuming a liquor:pulp
ratio of 3.7 l/kg = 3.6 litres

Table 4.29**Recovery and balance, hemicellulose, Cooks 29-31**

	Hemicellulose, % of original		
	Cook 29	Cook 30	Cook 31
H2SO4	0.05m	0.02m	0.01m
<u>Analyses</u>			
Removed in sampling	2	2	3
Pulp	0	5	27
Lignin	0	1	1
Liquor at cook end	16	54	68
Blown liquor	12	37	42
Liquor in chips	2	11	16
Filtered liquor	11	31	44
Final evaporated liquor	10	27	42
<u>Balance</u>			
Sampling, pulp, lignin	2	8	31
Liquor left in digester	2	6	8
Liquor in chips	2	11	16
Evaporated liquor	10	27	42
Losses:			
Cooking	82	39	2
Recovery of liquor from chips	0	0	2
Lignin precipitation	1	5	(-3)
Evaporation	1	4	2

Table 4.30

Recovery and balance, Klason lignin, Cooks 29-31

	-- Lignin, % of original --		
	Cook 29	Cook 30	Cook 31
H ₂ SO ₄	0.05m	0.02m	0.01m
<u>Analyses</u>			
Removed in sampling	3	3	3
Pulp	2	5	11
Liquor at cook end	94	90	84
Blown liquor	69	57	56
Liquor in chips	12	17	19
Filtered liquor	11	5	2
Precipitated lignin	53	40	34
<u>Balance</u>			
Sampling and pulp	5	8	14
Liquor left in digester	10	10	9
Liquor in chips	12	17	19
Filtered liquor	11	5	2
Recovered lignin	53	40	34
Losses:			
Cooking	1	2	2
Recovery of liquor from chips	3	6	0
Precipitation	5	12	20

Table 4.31

Recovery and balance, total solids, Cooks 29-31

	-- Solids, % of original --		
	Cook 29	Cook 30	Cook 31
H2SO4	0.05m	0.02m	0.01m
<u>Analyses</u>			
Removed in sampling	3	2	2
Pulp	32	47	55
Liquor at cook end	65	51	46
Blown liquor	47	35	30
Liquor in chips	9	10	10
Filtered liquor	30	22	20
Lignin	14	11	9
Final evaporated liquor	-	20	17
<u>Balance</u>			
Sampling, pulp and lignin	49	60	66
Liquor left in digester	7	6	5
Liquor in chips	9	10	10
Filtered liquor	30	-	-
Evaporated liquor	-	20	17
Losses:			
Cooking	0	0	(-3)
Recovery of liquor from chips	2	0	1
Lignin precipitation	3	2	1
Evaporation	-	2	3

Table 4.32

Cooking conditions and pulp analysis for 3-stage cooks

3 kg aspen chips cooked with 50:50 ethanol-water

	----- Cook Number -----			
	6	7-1	7-2	7-3
Volume of spent liquor*, l	-	12	6	-
Total liquor volume, l	30	7	24	24
Time to 190 deg C, min	55	55	28	22
Time at 190 deg C, min	30	5	5	5
pH at start	5.4	4.2	-	-
pH at cook end	4.1	4.0	4.1	4.1
Yield, %	-	-	-	51.6
Kappa No.	23.5	-	-	28.3
Viscosity, 0.5% CED, cp	39.2	-	-	35.6
Carbohydrates, %				
Xylan	-	-	-	7.8
Mannan	-	-	-	1.8
Glucan	-	-	-	82.7

* base spent liquor generated in Cook 6, readjusted to 50:50 EtOH-H₂O, total solids 28 g/l.

Table 4.33

Physical properties of 3-stage pulp

	CSF ml	PFI revs	Burst Index kPa.m2/g	Tear Index mN.m2/g	Tensile BL, km	Bulk cm3/g
Cook No. 6 single stage.	517	0	2.3	5.8	6.2	1.53
Extracted 0.5% NaOH	500	50	2.4	6.0	6.5	1.51
soln, 30 min, room temp	400	900	2.7	6.7	7.6	1.42
	300	3300	3.3	6.8	8.1	1.38
Cook No. 7, 3-stage.	536	0	2.4	6.0	5.6	1.60
Extracted 0.1% NaOH	500	50	2.7	6.2	6.3	1.56
soln, 30 min, room temp	400	1200	3.3	6.9	7.5	1.46
	300	4500	3.4	7.5	8.2	1.37

Table 5.1

Pulp	Pulp Yield %	----Strength at 300 ml CSF----		
		Tensile BL, km	Burst Index kPa.m2/g	Tear Index mN.m2/g
Single-stage organosolv	66	9.4	4.5	6.0
Sulfite	54	8.6	4.7	6.0
Kraft	57	10.2	5.7	8.0
3-stage organosolv	52	8.2	3.4	7.5

9.2 Appendix B

FIGURES

Figure 4.1

Variation of pH with Cooking Time
for Single-Stage Cooks 2-5

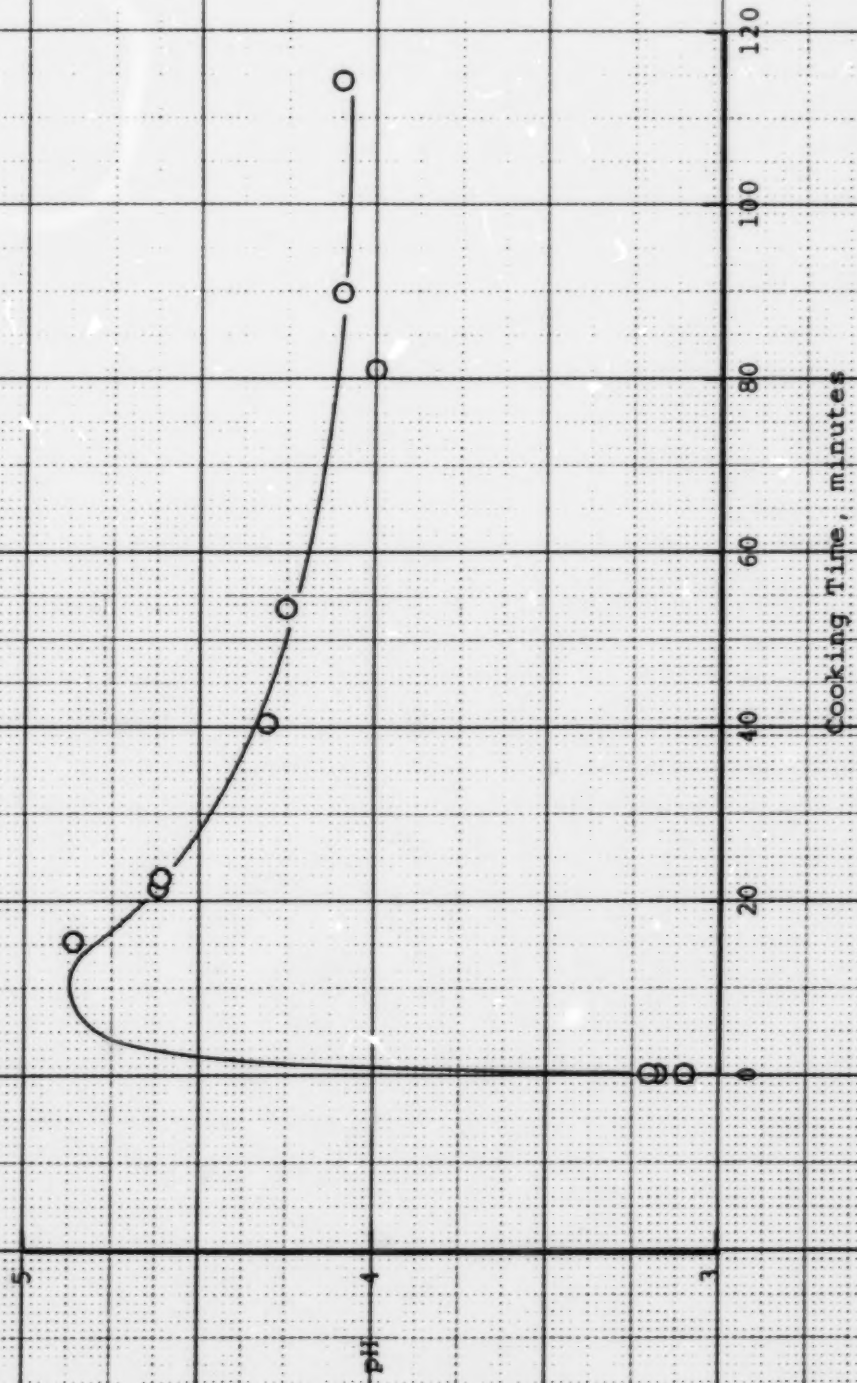
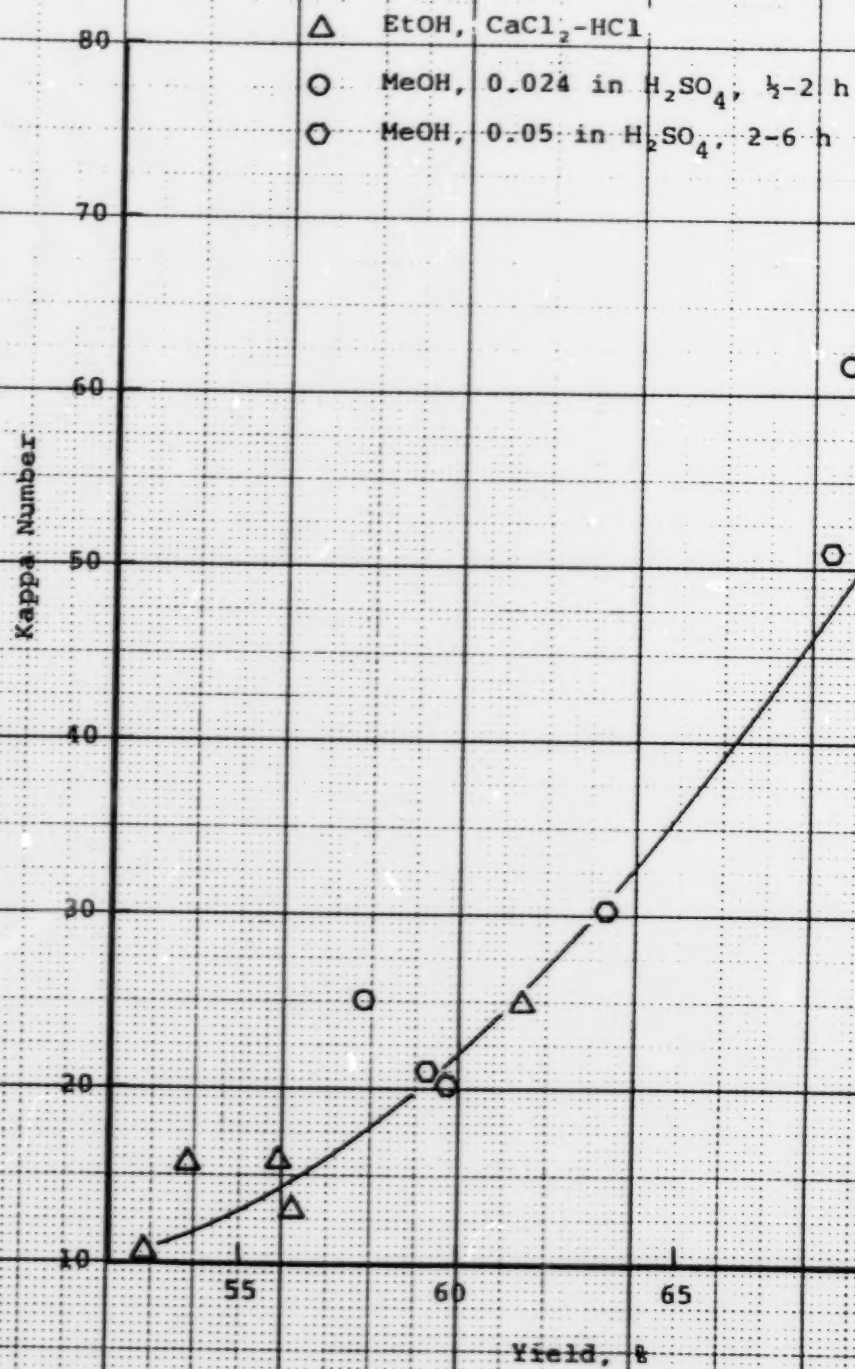
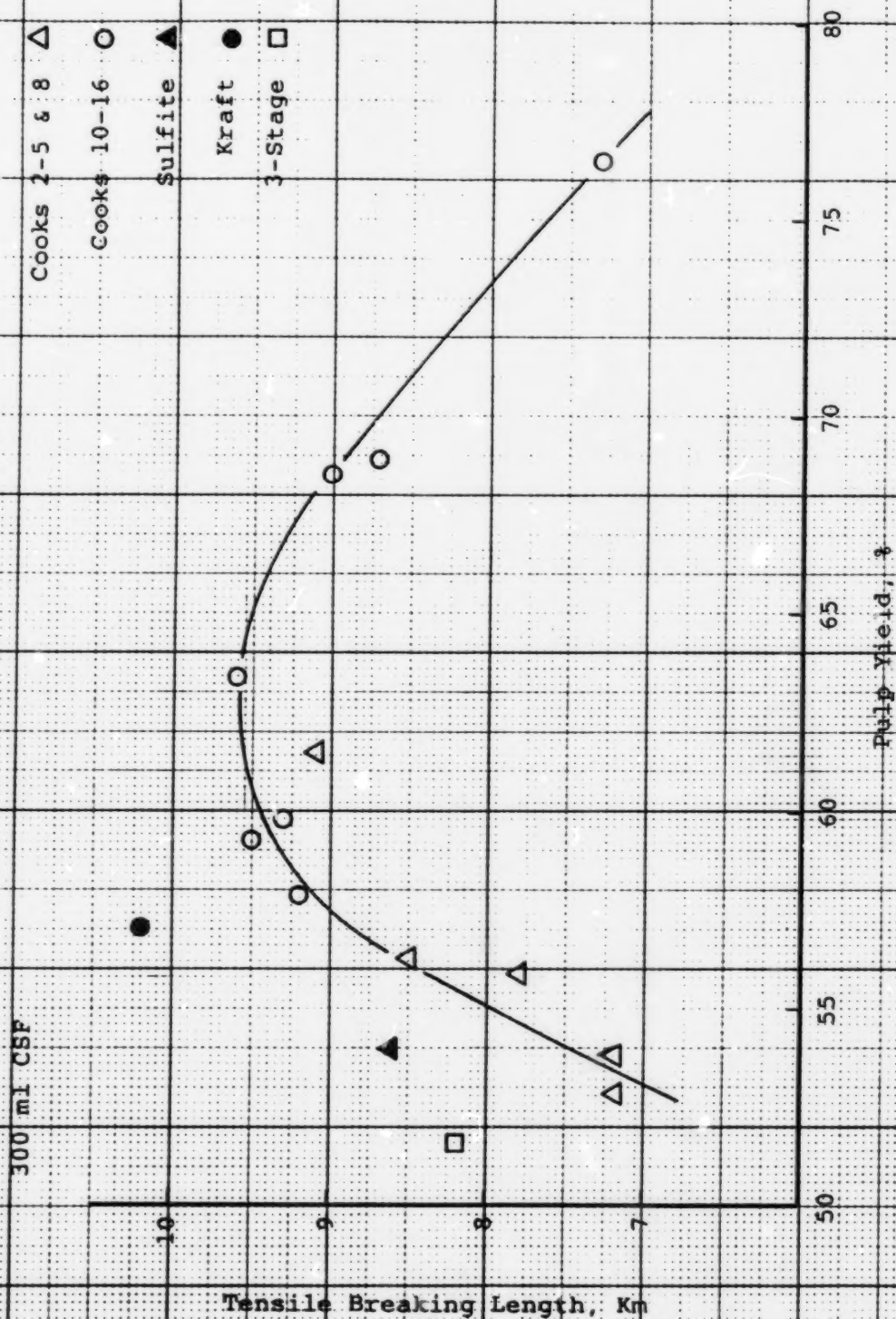


Figure 4.2
Kappa-Yield Relationship
for Single-Stage cooks



Effect of Pulp Yield on Tensile Strength of Single-Stage pulps
and comparison with Sulfite, Kraft and 3-Stage pulps



Effect of Pulp Yield on Burst Strength of Single-Stage Pulps and comparison with Sulfite, Kraft and 3-Stage pulps

300 ml CSF

Cooks 2-5 & 8 Δ
 Cooks 10-16 $\circ$
 Sulfite $\blacktriangle$
 Kraft $\bullet$
 3-Stage $\square$

Burst Index, $\text{kPa} \cdot \text{m}^2/\text{g}$

Pulp yield, %

Figure 4.4

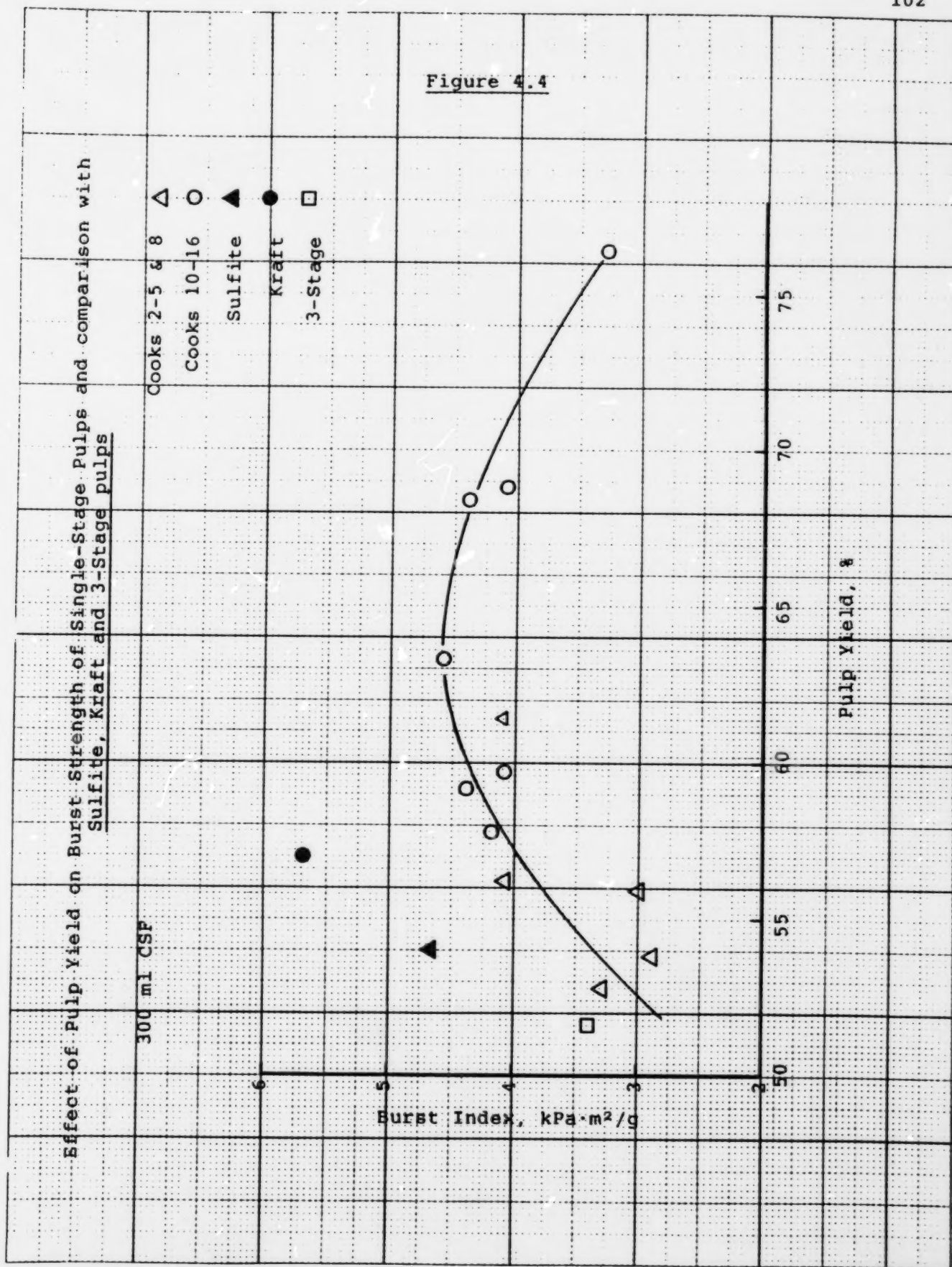


Figure 4.5

Effect of Pulp Yield on Tear Strength of Single-Stage pulps
and comparison with Sulfite, Kraft and 3-Stage pulps

300 ml CSF

Cooks 2-5 & B Δ
Cooks 10-16 $\circ$
Sulfite $\blacktriangle$
Kraft $\bullet$
3-Stage $\square$

Tear Index, $\text{mN}\cdot\text{m}^2/\text{g}$

Pulp Yield, %

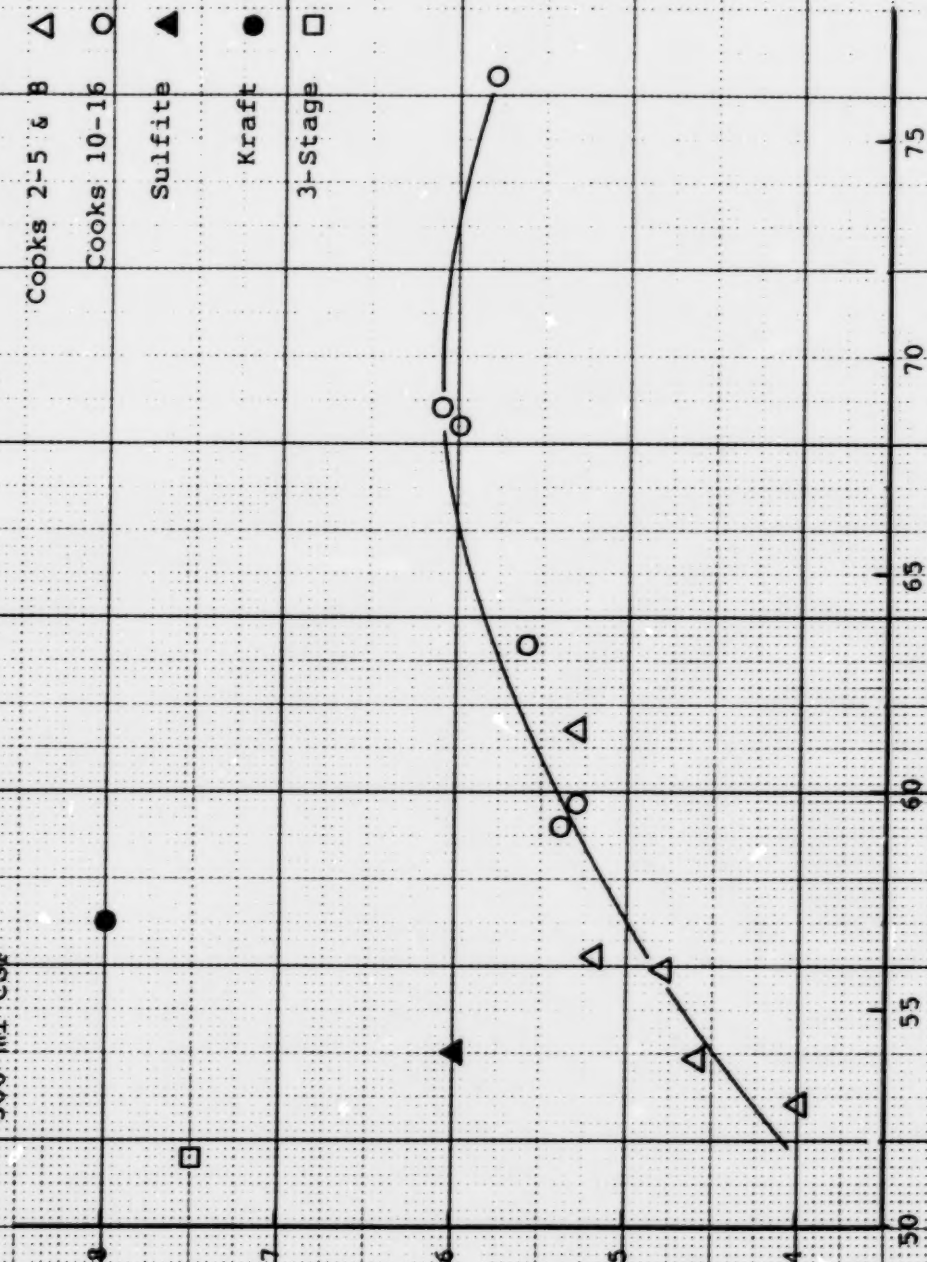


Figure 5.1
Effect of Pulp Yield on
Retention of Hemicellulose by pulp

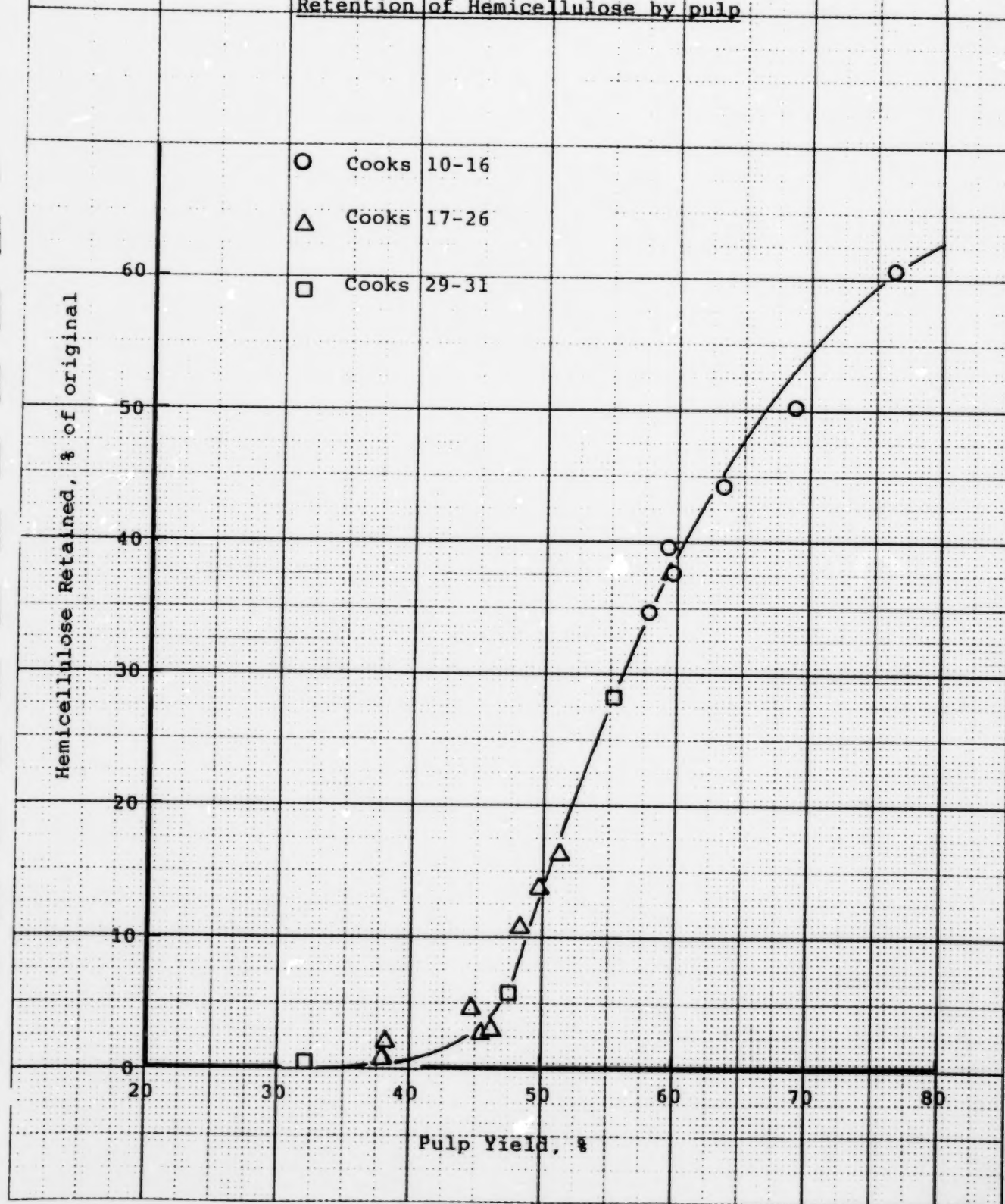
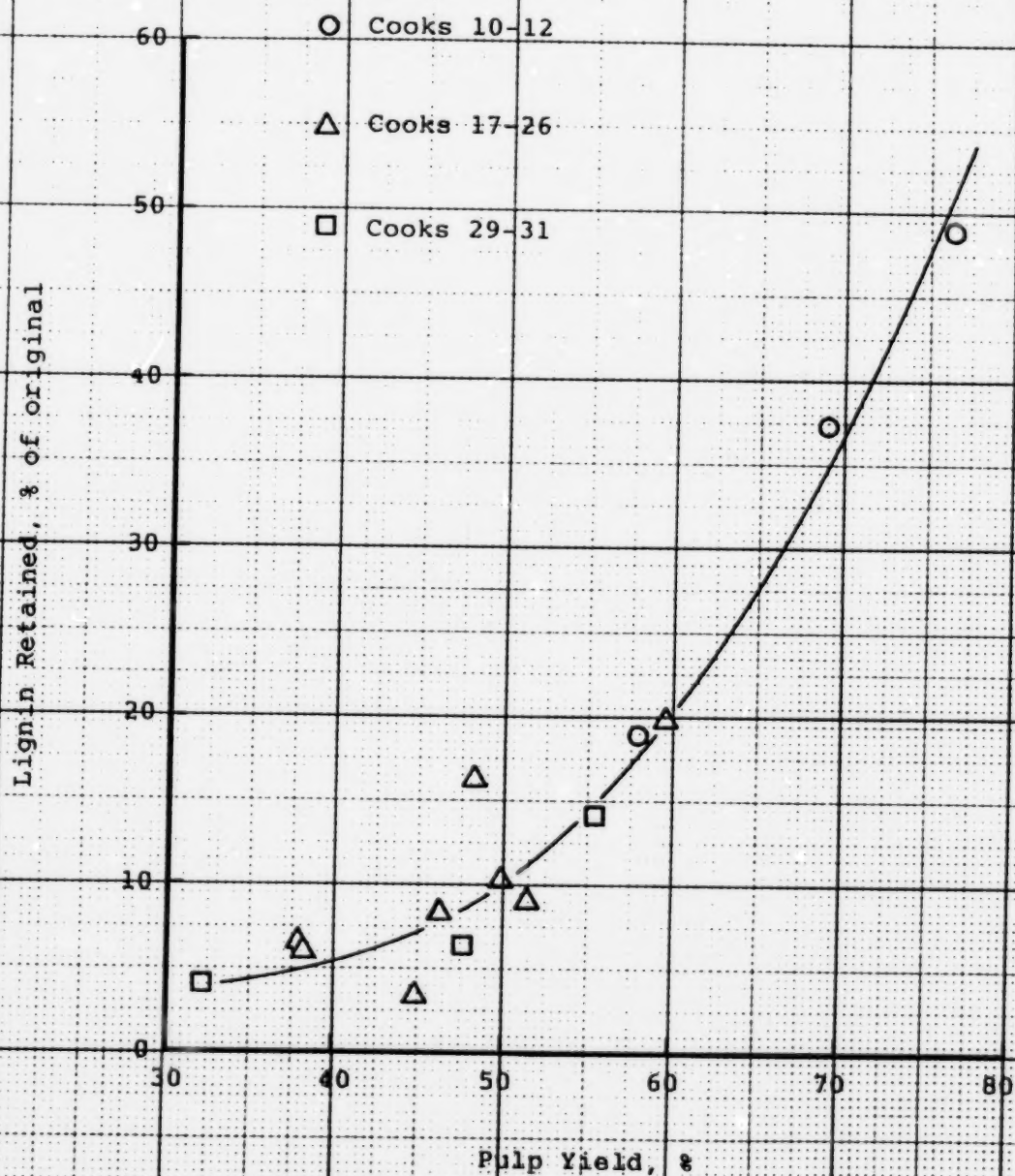


Figure 5.2
Effect of Pulp Yield on
Retention of Lignin by pulp



9.3 Appendix C

REPORT ON
"EVALUATION OF HEMICELLULOSE
FRACTIONS FOR FERMENTABILITY"

Phase I

Prepared for:

Econotech Services Ltd.
852 Derwent Way, Annacis Island
New Westminster, B.C.
V3M 5R1

Project 2-32-817

Prepared by:

Mr. E.G.-H. Lee
B.C. Research
3650 Wesbrook Mall
Vancouver, B.C.
V6S 2L2

May, 1985

- i -

TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	iv
OBJECTIVES	1
INTRODUCTION	1
MATERIALS AND METHODS	1
Source and Purpose of Cultures	1
Rehydration of Freeze-Dried Cultures	2
Maintenance of Cultures	2
Purity of Cultures	2
Inoculum Preparation	3
Synthetic Control Media Preparation	3
Fermentation Media Preparation	4
Fermentation Parameters	4
Sampling	5
Evaluation of Fermentation Performance	5
Analyses	6
- Acetone, Butanol, and Ethanol	6
- Biomass	6
- Sugars	6
- pH	6

- ii -

TABLE OF CONTENTS (Cont'd)

	<u>Page</u>
STAFF	7
RESULTS	7
Effect of Hydrolysis on Monomer Sugars Released from Hemicellulose	7
Fermentation of Synthetic Control Medium	7
Fermentability of Hemicellulose Hydrolysates	8
RECOMMENDATIONS FOR FUTURE WORK.	9
REFERENCES	11
LIST OF TABLES	iii
LIST OF FIGURES	iii
LIST OF APPENDICES	iii

- iii -

LIST OF TABLES

1. Monomer Sugars in Hemicellulose Fractions Before and After Acid Hydrolysis
2. Substrate Utilization by Various Cultures Grown on Synthetic Control Medium
3. Solvent Yields by Various Cultures Grown on Synthetic Control Medium
4. Fermentability of Hemicellulose Fractions Derived from Solvent Extraction of Wood

LIST OF FIGURES

1. Biomass Production on Synthetic Control Medium by Various Cultures
2. Solvent Production on Synthetic Control Medium by Various Cultures

LIST OF APPENDICES

- I (a) Fermentation on Synthetic Control Medium by Aerobic Cultures of Saccharomyces cerevisiae
- I (b) Fermentation on Synthetic Control Medium by Semi-aerobic Cultures of Saccharomyces cerevisiae
- II (a) Fermentation on Synthetic Control Medium by Aerobic Cultures of Pachysolen tannophilus
- II (b) Fermentation on Synthetic Control Medium by Semi-aerobic Cultures of Pachysolen tannophilus
- III Fermentation on Synthetic Control Medium by Clostridium acetobutylicum
- IV. Fermentability of Hemicellulose Fractions by Saccharomyces cerevisiae
- V Fermentability of Hemicellulose Fractions by Pachysolen tannophilus
- VI Fermentability of Hemicellulose Fractions by Clostridium acetobutylicum

- iv -

SUMMARY

The feasibility of producing ethanol, butanol, and acetone from hemicellulose hydrolysates was evaluated. The three cultures, Saccharomyces cerevisiae, Pachysolen tannophilus, and Clostridium acetobutylicum were tested for solvent production on synthetic medium. All three cultures grew and produced the expected solvents on the synthetic control medium.

No microbial growth and no fermentation activity was observed with any of the three cultures on as low as 10% concentration of hemicellulose hydrolysates. Evidently there are toxicants in the hemicellulose hydrolysates which inhibit growth.

To ferment hemicellulose hydrolysate removal of toxicants by some pretreatment procedures would be necessary to facilitate fermentation.

EVALUATION OF HEMICELLULOSE FRACTIONS FOR FERMENTABILITY

OBJECTIVES

- ° to test the fermentability of hemicellulose fractions derived from a solvent wood pretreatment process
- ° to determine whether the hemicellulose fractions are suitable for ethanol and acetone/butanol fermentations
- ° to determine whether there are any toxicants in the fractions that inhibit the fermentations.

INTRODUCTION

Econotech Services Ltd. submitted wood extract samples to B.C. Research for evaluation of the fermentability of the hemicellulose fractions to produce ethanol, butanol, and acetone, using Saccharomyces cerevisiae, Pachysolen tannophilus, and Clostridium acetobutylicum. This report describes the procedures used and the results obtained.

MATERIALS AND METHODS

Source and Purpose of Cultures

Freeze-dried cultures of

Saccharomyces cerevisiae ATCC 20201

Pachysolen tannophilus ATCC 2507 (NRRL Y2460)

Clostridium acetobutylicum NRC 2490 (ATCC 824)

were supplied by Roger K. Latta, Division of Biological Sciences,
National Research Council of Canada, Ottawa, Canada, K1A 0R6.

- 2 -

S. cerevisiae was used to convert hexoses to ethanol.

P. tannophilus was used to convert pentoses to ethanol.

Cl. acetobutylicum was used to produce butanol and acetone for wood sugars.

Rehydration of Freeze-Dried Cultures

The cultures were rehydrated according to the National Research Council's recommended procedures. Saccharomyces cerevisiae and Pachysolen tannophilus were transferred to yeast extract peptone, dextrose agar (YPDA), incubating at 25°C for 48 h. Clostridium acetobutylicum was transferred to Oxoid reinforced clostridial medium, incubating anaerobically for 2-3 days at 37°C.

Maintenance of Cultures

S. cerevisiae was maintained on dextrose, yeast extract, peptone (DYP) agar slants.

P. tannophilus was maintained on xylose, yeast extract, peptone (XYP) agar slants.

Cl. acetobutylicum was maintained on Oxoid reinforced clostridial agar slants.

All stock cultures were stored at 2°C for subsequent preparation of inoculum.

Purity of Cultures

The purity of the cultures was examined under the microscope and streaking on agar plates.

- 3 -

Inoculum Preparation

Inocula for fermentation experiments were prepared as follows:

S. cerevisiae (1 mL) was inoculated to 100 mL DYP medium,
P. tannophilus (1 mL) was inoculated to 100 mL XYP medium.

Both cultures were incubated aerobically in a gyratory shaker (100 rpm) at 30°C for 24 h.

Cl. acetobutylicum (1 mL) was transferred to 100 mL "Oxoid reinforced clostridial" broth and incubated under stationary anaerobic conditions (Forma Scientific model 1024 anaerobic chamber) for 48 h at 37°C.

For each experimental series, fresh inocula containing approximately 10^9 cells per mL were prepared.

Synthetic Control Media Preparation

Three media (coded A, B and C) containing the following ingredients were prepared for the three cultures:

Ingredients (g/L)	A	Media B	C
yeast extracts	1.5	1.5	5.0
peptone	2.0	2.0	2.0
dextrose	20	-	20
xylose	-	20	-
KH ₂ PO ₄	-	-	0.75
K ₂ H PO ₄	-	-	0.75
NH ₄ SO ₄	-	-	2.00
Mg SO ₄	-	-	0.2
MnSO ₄ ·7H ₂ O	-	-	0.01
FeSO ₄ ·7H ₂ O	-	-	0.01
cysteine hydrochloride	-	-	0.5

- 4 -

Medium A was used for S. cerevisiae

Medium B was used for P. tannophilus

Medium C was used for Cl. acetobutylicum

Fermentation Media Preparation

Hemicellulose extracts were hydrolyzed with 1N H₂SO₄ and autoclaved at 15 psi for 1 h. Three dilutions (v/v) of the hemicellulose hydrolysate of 10%, 50%, and 100% were made and nutrients were added as described in the synthetic control media. The media were sterilized by filtration by passing through a 0.45 micron Millipore filter.

Medium A and B were dispensed at 100 mL portions each, into 250 mL Erlenmeyer flasks and autoclaved at 15 psi for 15 min.

Medium C was boiled while flushing with N₂ and dispensed anaerobically in 100 mL aliquots into 250 mL Erlenmeyer flasks. The flasks were stoppered with rubber seals and autoclaved at 15 psi for 15 min.

Fermentation Parameters

S. cerevisiae and P. tannophilus were incubated in screw-cap flasks under stationary conditions at 28°C (1-3). Initial pH of the medium was 5.5. The two cultures were grown under "semi-aerobic" conditions, because screw caps were opened to the air and the cells were shaken by hand once a day during sampling.

- 5 -

C1. acetobutylicum was incubated at 37°C in the anaerobic chamber under stationary conditions (4,5). Initial pH of the medium was 6.5. This culture was kept inside of an anaerobic chamber throughout the experiment.

Sampling

Portions of 10 mL samples were taken from the cultures at the start and every 24 h up to 3 to 5 days of incubation. The samples were analyzed for biomass, pH, sugars, ethanol, acetone, and butanol. Samples from duplicate flasks were analyzed separately and duplicate results were recorded.

Evaluation of Fermentation Performance

The fermentability of the hemicellulose fractions were assessed in terms of:

- ° CO₂ evolution (as observed in the invert "gas vials")
- ° ethanol or acetone/butanol/ethanol concentration in the fermented broth
- ° utilization of hexoses and pentoses
- ° conversion efficiencies of carbohydrates to ethanol or acetone/butanol/ethanol.

The fermentability of the various substrates was compared and conclusions were drawn from the cultures' performance.

- 6 -

Analyses

Acetone, Butanol and Ethanol

Acetone, butanol and ethanol in the supernatant of the culture broth were analyzed by gas chromatography (Hewlett-Packard Model 5830A), with flame ionization detector (FID). Helium was used as carrier gas flowing through a stainless steel "Porapak Q" column (6 feet x 1/8" I.D.) at 30 mL/min at an initial temperature of 140°C for 5 min, then the temperature was increased to 210°C at 8°C/min.

Biomass

Biomass was determined by centrifuging an aliquot of 10 mL culture medium at 5000 rpm (3000 x G) for 10 min. The solid cake was then transferred to a 0.45 micron Millipore filter, rinsed with distilled water, dried at 105°C for 16 h and weighed.

Sugars

Xylose, glucose, galactose, mannose, and arabinose in the liquid medium before and after fermentation were analyzed by high performance liquid chromatography (HPLC) (6-9). A Hewlett Packard Model 1084A with refractive index detector was used. The sugars were separated by a 20 cm x 4.6 mm I.D. Lichrosorb-NH₂ (5µm) column, using water/acetonitrile : 20/80 (v/v) as mobile phase. Flow rate of the solvents was 2.5 mL/min.

pH

pH in fermentation media was measured by a Radiometer pH meter.

- 7 -

STAFF

This study was undertaken at B.C. Research by Mr. E.G-H. Lee, microbiologist; Mr. T. Vo, Mr. L. Chung and Dr. A.B. McKague, analysts.

Drs. J.C. Mueller and J. Howard assisted in report preparation and in future work recommendation for detoxification of organosolv pulping effluents to facilitate fermentation.

RESULTS

Effect of Hydrolysis on Monomer Sugars Released from Hemicellulose

The hemicellulose fractions were analyzed for monosaccharides before and after mild acid hydrolysis. The concentration of xylose in the hemicellulose fraction was increased by hydrolysis from 6.1 g/l to 28.6 g/l (Table 1). Glucose, galactose, mannose, and arabinose were present in small amounts ranging from 2.8 to 4 g/l (Table 1). The hydrolysis procedure evidently had been effective and had produced the desired pentose sugars.

Fermentation of Synthetic Control Medium

Saccharomyces cerevisiae, Pachysolen tannophilus and Clostridium acetobutylicum grew well and produced ethanol, butanol, and acetone (Figures 1 and 2) and produced gas.

After 2 to 3 days of fermentation Saccharomyces cerevisiae produced 4.9 g/l biomass and 4.5 g/l ethanol under continuous shaking at 100 rpm (Figures 1 and 2). Under semi-aerobic conditions S. cerevisiae produced only 2 g/l biomass and 4.4 g/l ethanol (Figure 1 and 2). Thus, less biomass but the same amount of ethanol were produced under limited oxygen conditions.

- 8 -

Aerated Pachysolen tannophilus cultures produced higher biomass but less ethanol than the semi-aerobic P. tannophilus cultures. After 5 days P. tannophilus produced 2.2 g/l biomass and 0.5 g/l ethanol aerobically; under semi-aerobic conditions 1.2 g/l biomass and 3.3 g/l ethanol were produced (Figures 1 and 2).

Clostridium acetobutylicum produced 2.2 g/l biomass and 3.2 g/l butanol, 1.2 g/l acetone, and 0.2 g/l ethanol under anaerobic conditions (Figures 1 and 2).

Under semi-aerobic conditions 100% glucose was utilized by S. cerevisiae and 89.1% xylose was used by P. tannophilus (Table 2). Under anaerobic conditions Clostridium acetobutylicum utilized 98.5% glucose (Table 2). The specific solvent yield expressed on the basis of sugar utilization varied from 0.18 to 0.23 g solvent produced per g monosaccharides removed (Table 3).

The experiments showed clearly, that under control conditions the cultures produced the desired solvents and proved that the procedures were correct. Hence, fermentation results on hemicellulose fractions could be compared against these control data.

Fermentability of Hemicellulose Hydrolysates

The specific concentrations of hemicellulose hydrolysates tested were 10%, 50% and 100%. Growth and fermentation yields were evaluated after 3 days of incubation.

No growth and no solvent production were observed on any of the hydrolysate media containing 10%, 50% and 100% hemicellulose hydrolysates (Table 4; Appendices IV to VI). Similarly, no growth and no solvent production were noted (data not shown) on untreated 10%, 50% and 100% hemicellulose media.

The results indicate that inhibitory or toxic substances are present in the hemicellulose hydrolysates.

- 9 -

RECOMMENDATIONS FOR FUTURE WORK

To make possible the fermentative utilization of hemicellulose hydrolysates for growth and solvent production, removal of toxicity will be an essential prerequisite. The following pretreatments should be considered as potential low cost methods of removing inhibitory substances from organosolv pulping effluent:

- Partial oxidation of the effluent

This could include a mild wet oxidation at $\sim 180 - 200^{\circ}\text{C}$; ie., the temperature used in the organosolv pulping process itself. Alternatively, the effluent at room temperature could be treated with hydrogen peroxide just sufficient to destroy toxicants. Both hydrogen peroxide and wet oxidation treatments will tend to destroy phenolic or lignin related materials and leave carbohydrates relatively unscathed.

- Treatment with Sacrificial Substrates

It is anticipated that high surface area materials such as activated charcoal, acid treated clays (bentonite, montmorillonite, etc.) and possibly some ion exchange resins will adsorb toxicants from organosolv pulping effluent. These substances probably are toxic simply because they are attracted to anionic enzyme surfaces. We should therefore offer them an alternative place to "plate out" before introducing organisms and enzymes to the system. These "sacrificial" surfaces could be regenerated after saturation. Charcoal can be re-activated by partial burning. Clays can also be regenerated, but it may not be economic. Ion exchange resins always have to be recovered because of their cost.

- 10 -

- Flocculation Using Lime, Alum, Iron

We should consider the possibility that toxicants are substances which may be in colloidal suspension and can be flocculated. We should determine whether pretreatment with lime followed by carbon dioxide to precipitate fresh calcium carbonate causes co-precipitation of toxicants. Alternatively we may try certain polyelectrolytes and ferrous sulphate or alum treatments as low cost detoxification methods.



E.G.-H. Lee
Principal Investigator
Fishery and Biotechnology



J.C. Mueller
Head, Fishery and Biotechnology

- 11 -

REFERENCES

1. Watson, N.E., Prior, B.A., du Preeze, J.C. and P.M. Lategan. 1984. Oxygen requirements for D-xylose fermentation to ethanol and polyols by Pachysolen tannophilus. Enzyme Micro. Tech. 6: 447-450.
2. Jeffries, T.W., Fady, J.H. and E.N. Lightfoot. 1984. Effect of glucose supplements on the fermentation of xylose by Pachysolen tannophilus. Biotech. and Bioeng. 27:171-176.
3. Mahmoudides, G., Lee, H., Maki, N. and H. Schneider. 1985. Ethanol accumulation in cultures of Pachysolen tannophilus on D-xylose is associated with a transition to a state of low oxygen consumption. Biotech 1:59-62.
4. Mes-Hartree, M. and J.N. Saddler. 1982. Butanol production of Clostridium acetobutylicum grown on sugars found in hemicellulose hydrolysates. Biotech. Lett. 4:247-252.
5. Saddler, J.N., Yu, E.K.C., Mes-Hartree, M., Levitin N., and H.H. Brownell. 1983. Utilization of enzymatically hydrolyzed wood hemicelluloses by microorganisms for production of liquid fuels. Appl. Environ. Microbiol. 45:153-160.
6. Wentz, F.E., Marcy, A.D., and M.J. Gray. 1982. Analysis of wood sugars in pulp and paper industry samples by HPLC. J. Chromatogr. Sci. 20:349-352.
7. Paice, M.G., Jurasek, L. and M. Desrochers. 1982. Simplified analysis of wood sugars. Tappi 65(7): 103-106.
8. Götz, H. 1982. Analysis of Carbohydrates in Food in HPLC. Hewlett-Packard Application Note AN 232-14.

- 12 -

9. Tweeten, T.N. and C.B. Euston. 1980. Application of high performance liquid chromatography in the food industry. Food Technology 12:29-37.

TABLE 1
Monomer Sugars In Hemicellulose
Fractions Before and After Acid Hydrolysis

Carbohydrates	Hemicellulose* (no hydrolysis)	Hemicellulose Hydrolysate**
Xylose (g/L)	6.1	28.6
Glucose (g/L)	0.7	3.2
Galactose (g/L)	0.5	3.1
Mannose (g/L)	0.6	4.0
Arabinose (g/L)	0.9	2.8

* Ethanol wood extract from Cook #5 batch (3.3% residue ethanol); data supplied by Econotech.

** Wood extract hydrolyzed with 1N H₂SO₄ at 15 psi for 1 h.

TABLE 2
Substrate Utilization by
Various Cultures Grown on Synthetic Control Medium

CULTURES	SUBSTRATE						REFERENCES APPENDIX
	Glucose			Xylose			
	Start (g/l)	End (g/l)	Removed (%)	Start (g/l)	End (g/l)	Removed (%)	
<u>S. cerevisiae</u> (semi-aerobic)	20.2	0	100	-	-	-	I(b)
<u>P. tannophilus</u> (semi-aerobic)	-	-	-	20.1	2.2	89.1	II(b)
<u>Cl. acetobutylicum</u> (anaerobic)	20.1	0.3	98.5	-	-	-	III

TABLE 3
Solvent Yields By Various Cultures
Grown On Synthetic Control Medium

Cultures	Carbohydrates Used (g/l)	Solvents Produced (g/l)	Solvent Yields* (%)	Reference Appendix
<u>S. cerevisiae</u> (semi-aerobic)	20.2	4.0	20**	I(b)
<u>P. tannophilus</u> (semi-aerobic)	17.9	3.3	18**	II(b)
<u>C. acetobutylicum</u> (anaerobic)	19.8	4.6	23***	III

* % Solvent yields = $\frac{\text{Solvents produced (g/l)}}{\text{Carbohydrates used (g/l)}} \times 100$

** Ethanol

*** Total solvents = butanol, acetone, and ethanol

TABLE 4
Fermentability of Hemicellulose Fractions
Derived from Solvent Extraction of Wood

Substrate	Hemicellulose Hydrolysate Concentration (%)	Cultures	Growth*	Solvent Production*
Glucose or Xylose (control synthetic medium)	n/a	<u>S. cerevisiae</u>	Yes	Yes
		<u>P. tannophilus</u>	Yes	Yes
		<u>Cl. acetobutylicum</u>	Yes	Yes
	10%	<u>S. cerevisiae</u>	No	No
		<u>P. tannophilus</u>	No	No
		<u>Cl. acetobutylicum</u>	No	No
Hemicellulose Hydrolysate	50%	<u>S. cerevisiae</u>	No	No
		<u>P. tannophilus</u>	No	No
		<u>Cl. acetobutylicum</u>	No	No
	100%	<u>S. cerevisiae</u>	No	No
		<u>P. tannophilus</u>	No	No
		<u>Cl. acetobutylicum</u>	No	No

* Appendices IV to VI

FIGURE 1.
BIOMASS PRODUCTION ON SYNTHETIC CONTROL MEDIUM
BY VARIOUS CULTURES

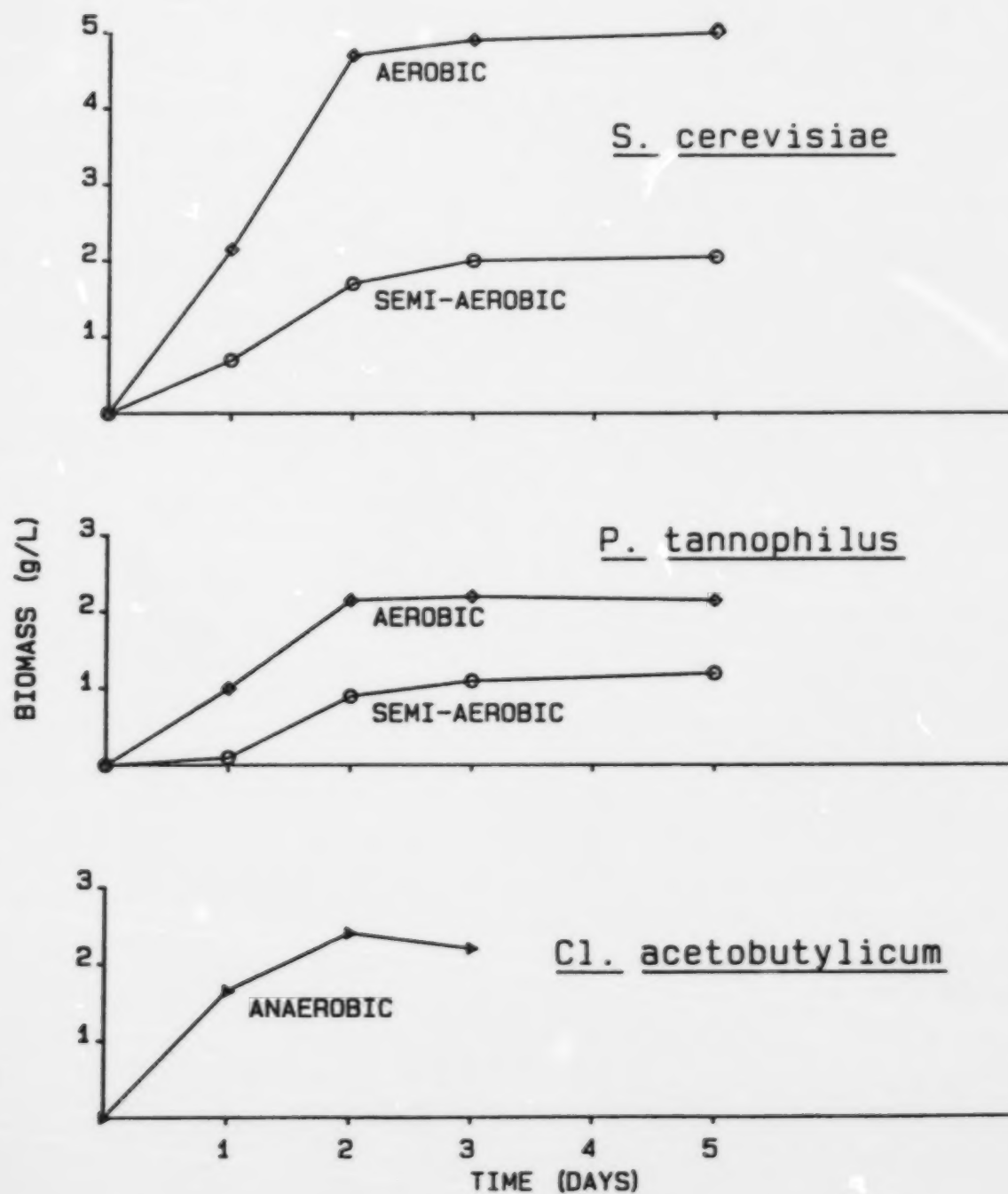
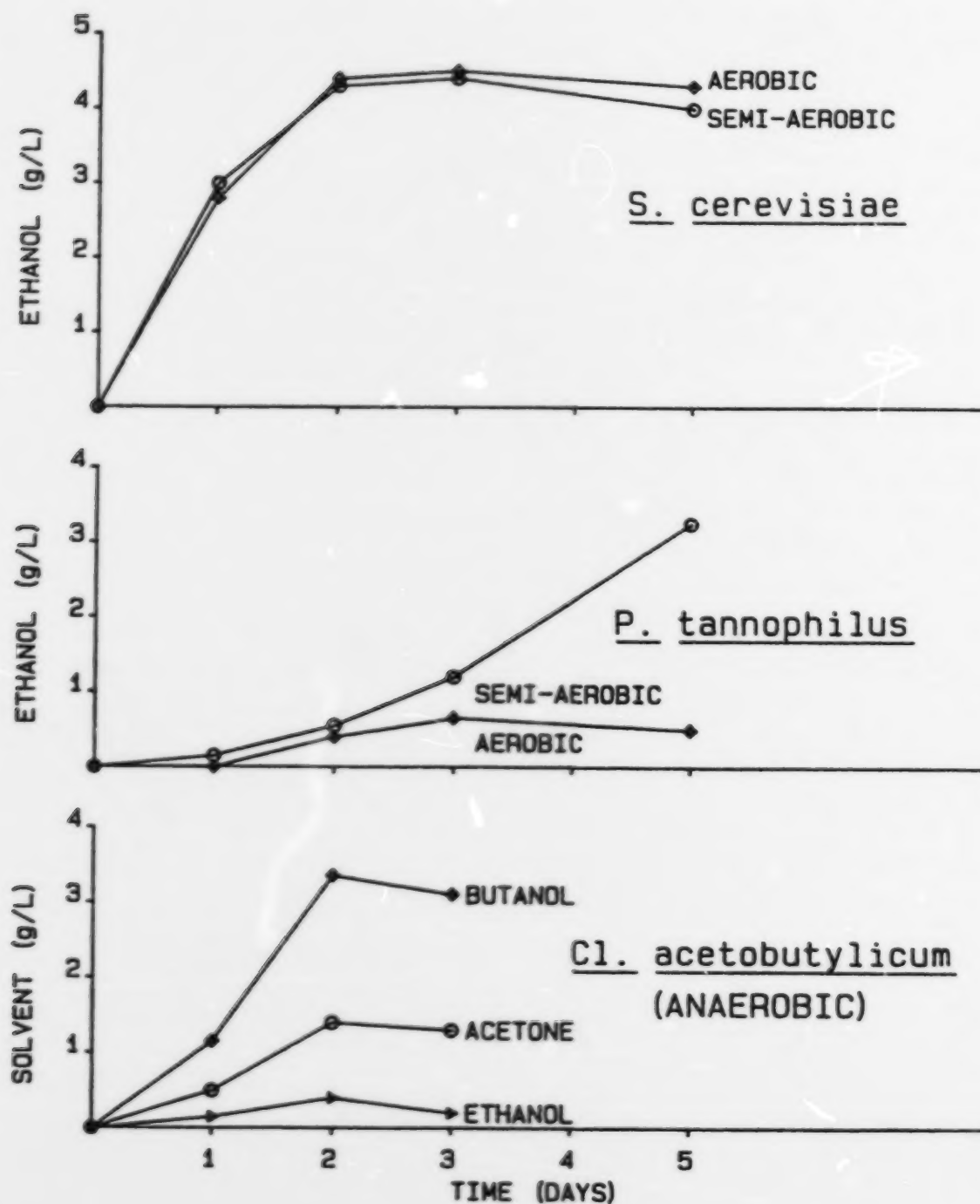


FIGURE 2.
SOLVENT PRODUCTION ON SYNTHETIC CONTROL MEDIUM
BY VARIOUS CULTURES



Appendix I(a)

Fermentation On Synthetic Control Medium By

Aerobic Cultures of Saccharomyces cerevisiae

Fermentation Conditions: - 1% inoculum; 24 h aerobic culture
 - batch fermentation (100 mL medium in 250 mL
 cotton-plugged Erlenmeyer flask)
 - shaking at 100 rpm; 28°C.

Incubation time (h)	Run #	pH	Biomass (g/L)	Glucose (g/L)	Ethanol (g/L)
Start	1	5.5	0.02	20.2	0
	2	5.5	0.02	20.2	0
	Average	5.5	0.02	20.2	0
24	1	3.5	2.1	5.1	2.6
	2	3.4	2.2	4.9	2.9
	Average	3.5	2.2	5.0	2.8
48	1	2.8	4.6	0	4.3
	2	2.9	4.8	0	4.5
	Average	2.9	4.7	0	4.4
72	1	2.8	4.8	0	4.5
	2	2.9	4.9	0	4.5
	Average	2.9	4.9	0	4.5
120	1	2.8	5.2	0	4.2
	2	2.7	5.0	0	4.3
	Average	2.8	5.1	0	4.3

Appendix I(b)

Fermentation On Synthetic Control Medium By

Semi-aerobic Cultures of Saccharomyces cerevisiae*

Fermentation Conditions: - 1% inoculum; 24 h aerobic culture
 - batch fermentation (100 mL medium in 500 mL screw-capped Erlenmeyer flask)
 - stationary at 28°C; flask was opened once per day

Incubation time (h)	Run #	pH	Biomass (g/L)	Glucose (g/L)	Ethanol (g/L)
Start	1	5.5	0.02	20.2	0
	2	5.5	0.02	20.2	0
	Average	5.5	0.02	20.2	0
24	1	4.5	0.6	11.1	2.9
	2	4.2	0.7	10.2	3.1
	Average	4.4	0.7	10.7	3.0
48	1	3.2	1.6	6.2	4.1
	2	2.9	1.8	5.3	4.5
	Average	3.1	1.7	5.8	4.3
72	1	3.0	1.9	1.0	4.3
	2	2.9	2.1	0.8	4.5
	Average	3.0	2.0	0.9	4.4
120	1	3.0	2.1	0	3.9
	2	2.9	2.1	0	4.1
	Average	3.0	2.1	0	4.0

* Gas produced at 16h in an invert "gas vial" in a test tube containing 10 ml medium.

Appendix II(b)

Fermentation On Synthetic Control Medium By

Semi-aerobic Cultures of Pachysolen tannophilus*

Fermentation Conditions: - 1% inoculum; 24 h aerobic culture
 - batch fermentation (100 mL medium in 500 mL screw-capped Erlenmeyer flask)
 - stationary at 28°C.

Incubation time (h)	Run #	pH	Biomass (g/L)	Xylose (g/L)	Ethanol (g/L)
Start	1	5.5	0.01	20.1	0
	2	5.5	0.01	20.1	0
	Average	5.5	0.01	20.1	0
24	1	4.7	0.1	17.2	0.1
	2	4.8	0.1	18.5	0.1
	Average	4.8	0.1	17.9	0.1
48	1	3.8	0.8	13.5	0.7
	2	3.9	0.9	12.9	0.9
	Average	3.9	0.9	13.2	0.6
72	1	2.8	1.0	10.4	1.1
	2	3.0	1.2	9.8	1.2
	Average	2.9	1.1	10.1	1.2
120	1	2.8	1.1	2.1	3.1
	2	2.9	1.2	2.3	3.4
	Average	2.9	1.2	2.2	3.3

* Gas produced at 48 h in an invert "gas vial" in a test tube containing 10 ml medium.

Appendix II(a)

Fermentation On Synthetic Control Medium By

Aerobic Cultures of Pachysolen tannophilus

Fermentation Conditions: - 1% inoculum; 24 h aerobic culture
 - batch fermentation (100 mL medium in 250 mL cotton-plugged Erlenmeyer flask)
 - shaking at 100 rpm; at 28°C.

Incubation time (h)	Run #	pH	Biomass (g/L)	Xylose (g/L)	Ethanol (g/L)
Start	1	5.5	0.01	20.1	0
	2	5.5	0.01	20.1	0
	Average	5.5	0.01	20.1	0
24	1	4.2	0.9	11.3	0
	2	4.5	1.1	12.1	0
	Average	4.4	1.0	11.7	0
48	1	3.9	2.0	1.0	0.5
	2	3.9	2.2	1.2	0.3
	Average	3.9	2.1	1.1	0.4
72	1	3.5	2.1	0	0.6
	2	3.7	2.2	0	0.7
	Average	3.6	2.2	0	0.7
120	1	3.1	2.1	0	0.4
	2	3.1	2.3	0	0.5
	Average	3.1	2.2	0	0.5

Appendix III

Fermentation On Synthetic Control Medium By

Clostridium acetobutylicum*

Fermentation Conditions: - 1% inoculum; (48 h anaerobic culture).
 - batch fermentation; 100 mL in Erlenmeyer flask.
 - stationary in anaerobic chamber at 37°C.

Incubation time (h)	Run #	pH	Biomass (g/L)	Glucose (g/L)	Butanol (g/L)	Acetone (g/L)	Ethanol (g/L)
Start	1	6.5	0.02	20.1	0	0	0
	2	6.5	0.02	20.1	0	0	0
	Average	6.5	0.02	20.1	0	0	0
24	1	5.5	1.5	12.5	1.1	0.4	0.1
	2	5.6	1.7	11.2	1.2	0.5	0.1
	Average	5.6	1.6	11.9	1.2	0.5	0.1
48	1	4.4	2.2	0.5	3.2	1.2	0.3
	2	4.2	2.5	0.6	3.5	1.4	0.4
	Average	4.3	2.4	0.6	3.4	1.3	0.4
72	1	4.1	2.1	0.2	3.0	1.1	0.2
	2	4.0	2.3	0.3	3.4	1.2	0.2
	Average	4.1	2.2	0.3	3.2	1.2	0.2

* Gas produced at 16 h in an invert "gas vial" in a test tube containing 10 ml medium.

Appendix IV

Fermentability of Hemicellulose Fractions By *Saccharomyces cerevisiae*

Fermentation Conditions: - 1% inoculum.
 - batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
 - stationary at 28°C.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Xylose (g/L)	Glucose (g/L)	Galactose (g/L)	Mannose (g/L)	Arabinose (g/L)	Ethanol (g/L)
Glucose, yeast extract, and peptone (control medium)	start	1	-	5.5	0.02	0	20.2	0	0	0	0
		2	-	5.5	0.02	0	20.2	0	0	0	0
	72	Average	-	5.5	0.02	0	20.2	0	0	0	0
		1	+	2.8	2.4	0	1.2	0	0	0	4.5
	Average	2	+	2.9	2.2	0	0.7	0	0	0	4.0
10% hemicellulose hydrolysate	start	1	-	5.5	0.02	2.9	0.32	0.2	0.39	0.25	0.05
		2	-	5.5	0.02	2.9	0.32	0.2	0.39	0.25	0.05
	72	Average	-	5.5	0.02	2.9	0.32	0.2	0.39	0.25	0.05
		1	-	5.6	0	-	-	-	-	-	0.05
	Average	2	-	5.5	0	-	-	-	-	-	0.05
50% hemicellulose hydrolysate	start	1	-	5.5	0.02	-	-	-	-	-	-
		2	-	5.5	0.02	-	-	-	-	-	-
	72	Average	-	5.5	0.02	-	-	-	-	-	-
		1	-	5.8	0	-	-	-	-	-	-
	Average	2	-	5.6	0	-	-	-	-	-	-
100% hemicellulose hydrolysate	start	1	-	5.5	0.02	-	-	-	-	-	-
		2	-	5.5	0.02	-	-	-	-	-	-
	72	Average	-	5.5	0.02	-	-	-	-	-	-
		1	-	5.8	0	-	-	-	-	-	-
	Average	2	-	5.7	0	-	-	-	-	-	-

Appendix V

Fermentability of Hemicellulose Fractions By *Pachysolen tannophilus*

Fermentation Conditions: - 1% inoculum.
 - batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
 - stationary at 28°C.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Xylose (g/L)	Glucose (g/L)	Galactose (g/L)	Mannose (g/L)	Arabinose (g/L)	Ethanol (g/L)
Xylose, yeast extract, and peptone (control medium)	start	1	-	5.5	0.01	20.0	0	0	0	0	0
		2	-	5.5	0.01	20.1	0	0	0	0	0
	Average			5.5	0.01	20.1	0	0	0	0	0
	72	1	+	3.0	1.1	9.5	0	0	0	0	0.9
		2	+	2.9	1.2	9.1	0	0	0	0	1.1
	Average		+	3.0	1.2	9.3	0	0	0	0	1.0
10% hemicellulose hydrolysate	start	1	-	5.5	0.01	2.9	0.32	0.2	0.39	0.25	0.05
		2	-	5.5	0.01	2.9	0.32	0.2	0.39	0.25	0.05
	Average			5.5	0.01	2.9	0.32	0.2	0.39	0.25	0.05
	72	1	-	5.6	0	-	-	-	-	-	0.05
		2	-	5.5	0	-	-	-	-	-	0.05
	Average		-	5.6	0	-	-	-	-	-	0.05
50% hemicellulose hydrolysate	start	1	-	5.5	0.01	-	-	-	-	-	-
		2	-	5.5	0.01	-	-	-	-	-	-
	Average			5.5	0.01	-	-	-	-	-	-
	72	1	-	5.8	0	-	-	-	-	-	-
		2	-	5.6	0	-	-	-	-	-	-
	Average		-	5.7	0	-	-	-	-	-	-
100% hemicellulose hydrolysate	start	1	-	5.5	0.01	-	-	-	-	-	-
		2	-	5.5	0.01	-	-	-	-	-	-
	Average			5.5	0.01	-	-	-	-	-	-
	72	1	-	5.6	0	-	-	-	-	-	-
		2	-	5.7	0	-	-	-	-	-	-
	Average		-	5.7	0	-	-	-	-	-	-

Appendix VI

Fermentability of Hemicellulose Fractions By Clostridium acetobutylicum

Fermentation Conditions: - 1% inoculum.

- batch fermentation; (10 mL medium in a test tube containing invert "gas vial").

- stationary in anaerobic chamber at 37°C.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Xylose (g/L)	Glucose (g/L)	Galactose (g/L)	Mannose (g/L)	Arabinose (g/L)	Butanol (g/L)	Acetone (g/L)	Ethanol (g/L)
Glucose (control medium)	72	start	-	6.5	0.02	0	20.0	0	0	0	0	0	0
		1	-	6.5	0.02	0	20.0	0	0	0	0	0	0
		2	-	6.5	0.02	0	20.0	0	0	0	0	0	0
		Average	-	6.5	0.02	0	20.0	0	0	0	0	0	0
		1	+	3.9	2.6	0	0.5	0	0	0	1.0	0.4	0.1
10% Hemicellulose hydrolysate	72	2	+	4.1	2.1	0	0.2	0	0	0	0.8	0.3	0.1
		Average	+	4.0	2.4	0	0.4	0	0	0	0.9	0.4	0.1
		start	-	6.5	0.02	2.9	0.35	0.3	0.38	0.29	0	0	0.05
		1	-	6.5	0.02	2.9	0.35	0.3	0.38	0.29	0	0	0.05
		Average	-	6.5	0	-	-	-	-	-	-	-	-
50% Hemicellulose hydrolysate	72	2	-	6.5	0	-	-	-	-	-	-	-	-
		Average	-	6.5	0	-	-	-	-	-	-	-	-
		start	-	6.5	0.02	-	-	-	-	-	-	-	-
		1	-	6.5	0.02	-	-	-	-	-	-	-	-
		Average	-	6.5	0	-	-	-	-	-	-	-	-
100% Hemicellulose hydrolysate	72	2	-	6.5	0	-	-	-	-	-	-	-	-
		Average	-	6.5	0	-	-	-	-	-	-	-	-
		start	-	6.5	0.02	-	-	-	-	-	-	-	-
		1	-	6.5	0.02	-	-	-	-	-	-	-	-
		Average	-	6.5	0	-	-	-	-	-	-	-	-

9.4 Appendix D

REPORT ON
"EVALUATION OF ORGANOSOLV
SPENT LIQUOR
FOR FERMENTABILITY"

Phase II

Prepared for:

Econotech Services Ltd.
852 Derwent Way, Annacis Island
New Westminster, B.C.
V3M 5R1

Project 2-32-817

Prepared by:

Mr. E.G-H. Lee
B.C. Research
3650 Wesbrook Mall
Vancouver, B.C.
V6S 2L2

November, 1985

- i -

TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	v
OBJECTIVES	1
INTRODUCTION	1
MATERIALS AND METHODS.	1
Inoculum Preparation	1
Synthetic Basal Media Composition	2
Preparation of Media	2
Fermentation Parameters	3
Sampling	3
Evaluation of Fermentation Performance	3
Analyses	4
- Acetone, butanol, and ethanol	4
- Biomass	4

- ii -

TABLE OF CONTENTS (Cont'd)

	<u>Page</u>
- Sugars	4
- pH	5
STAFF	5
RESULTS	5
REFERENCES	7
LIST OF TABLES	iii
LIST OF APPENDICES	iv

- iii -

LIST OF TABLESTable

1. Fermentability of Organosolv Spent Liquor (OSL)
2. Ethanol Yields By S. cerevisiae Grown on Organosolv Spent Liquor (OSL)
3. Ethanol Yields By P. tannophilus Grown on Organosolv Spent Liquor (OSL)
4. Solvent Yields By Cl. acetobutylicum Grown on Organosolv Spent Liquor (OSL)

- iv -

LIST OF APPENDICESAppendix

- I(a) Fermentability of Untreated Organosolv Spent Liquor
(OSL) By Saccharomyces cerevisiae
- I(b) Fermentability of Hydrolyzed Organosolv Spent Liquor
(OSL) By Saccharomyces cerevisiae
- II(a) Fermentability of Untreated Organosolv Spent Liquor
(OSL) By Pachysolen tannophilus
- II(b) Fermentability of Hydrolyzed Organosolv Spent Liquor
(OSL) by Pachysolen tannophilus
- III(a) Fermentability of Untreated Organosolv Spent Liquor
(OSL) by Clostridium acetobutylicum
- III(b) Fermentability of Hydrolyzed Organosolv Spent Liquor
(OSL) by Clostridium acetobutylicum

- v -

SUMMARY

Three cultures, Saccharomyces cerevisiae, Pachysolen tannophilus, and Clostridium acetobutylicum were used to evaluate growth and solvent production on synthetic media containing various amounts of organosolvent liquor (OSL). All three cultures grew well and produced solvent at 5% OSL concentration. Two cultures, S. cerevisiae and P. tannophilus grew and produced ethanol at 10% OSL; however Clostridium acetobutylicum was suppressed in growth and solvent production. No microbial growth and no fermentation activity were observed with any of the three cultures at or above 25% OSL addition to the synthetic medium.

OBJECTIVES

- To evaluate growth and solvent production on synthetic media containing various concentrations of organosolv spent liquor (OSL).
- To identify toxicity levels of OSL to the cultures.

INTRODUCTION

Econotech Services Ltd. submitted wood extract samples to B.C. Research for evaluation of the fermentability of the organosolv spent liquor (OSL) to produce ethanol, butanol, and acetone, using Saccharomyces cerevisiae, Pachysolen tannophilus, and Clostridium acetobutylicum. This report describes the procedures used and the results obtained.

MATERIALS AND METHODS

Inoculum Preparation

Inocula for fermentation experiments were prepared as follows:

- S. cerevisiae (1 mL) was inoculated to 100 mL DYP medium,
- P. tannophilus (1 mL) was inoculated to 100 mL XYP medium.

Both cultures were incubated aerobically in a gyratory shaker (100 rpm) at 30°C for 24 h.

Cl. acetobutylicum (1 mL) was transferred to 100 mL "Oxoid reinforced clostridial" broth and incubated under stationary anaerobic conditions (Forma Scientific model 1024 anaerobic chamber) for 48 h at 37°C.

For each experimental series, fresh inocula containing approximately 10^9 cells per mL were prepared.

- 2 -

Synthetic Basal Media Composition.

Three media (coded A, B and C) containing the following ingredients were prepared for the three cultures:

Ingredients (g/L)	Media		
	A	B	C
yeast extracts	1.5	1.5	5.0
peptone	2.0	2.0	2.0
dextrose	20	-	20
xylose	-	20	-
KH_2PO_4	-	-	0.75
K_2HPO_4	-	-	0.75
NH_4Cl	-	-	2.00
MgSO_4	-	-	0.2
$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$	-	-	0.01
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	-	-	0.01
cysteine hydrochloride	-	-	0.5

Medium A was used for S. cerevisiae

Medium B was used for P. tannophilus

Medium C was used for Cl. acetobutylicum

Preparation of Media

A portion of the organosolv spent liquor (OSL) was hydrolyzed with 1N H_2SO_4 and autoclaved at 15 psi for 1 h. Suspended solids were removed by centrifugation. Supernatant was neutralized with NaOH and used as hydrolyzed OSL.

- 3 -

Media "A", "B" and "C" were prepared and five specific concentrations (0.1, 1, 5, 10, and 25%; v/v) of untreated and hydrolyzed OSL were added. Ten milliliter aliquots of each medium were dispensed into screw-cap test tubes containing invert "gas vials". All media were autoclaved at 15 psi for 15 min. To minimize dissolved oxygen in medium "C", test tubes were transported into an anaerobic chamber immediately after autoclaving.

Fermentation Parameters

S. cerevisiae and P. tannophilus were incubated in screw-cap test tubes under stationary conditions at 28°C. Initial pH of the medium was 6.1.

Cl. acetobutylicum was incubated at 37°C in the anaerobic chamber under stationary conditions. Initial pH of the medium was 6.5. This culture was kept inside an anaerobic chamber throughout the experiment.

Sampling

Two 10 mL samples were used from the cultures at the start and after 3 days of incubation. The samples were analyzed for biomass, pH, sugars, ethanol, acetone, and butanol. Samples from duplicate tubes were analyzed separately and duplicate results were recorded.

Evaluation of Fermentation Performance

The fermentability of the hemicellulose fractions was assessed in terms of:

- CO₂ evolution (as observed in the invert "gas vials")
- ethanol or acetone/butanol/ethanol concentration in the fermented broth

- 4 -

- utilization of hexoses and pentoses
- conversion efficiencies of carbohydrates to ethanol or acetone/butanol/ethanol.

The fermentability of the various substrates was compared and conclusions were drawn from the cultures' performance.

Analyses

Acetone, butanol and ethanol

Acetone, butanol and ethanol in the supernatant of the culture broth were analyzed by gas chromatography (Hewlett-Packard Model 5830A), with flame ionization detector (FID). Helium was used as carrier gas flowing through a stainless steel "Porapak Q" column (6 feet x 1/8" I.D.) at 30 mL/min at an initial temperature of 140°C for 5 min, then the temperature was increased to 210°C at 8°C/min.

Biomass

Biomass was determined by centrifuging an aliquot of 10 mL culture medium at 5000 rpm (3000 x G) for 10 min. The solid cake was then transferred to a 0.45 micron Millipore filter, rinsed with distilled water, dried at 105°C for 16 h and weighed.

Sugars

Xylose and glucose in the liquid medium before and after fermentation were analyzed by high performance liquid chromatography (HPLC) (1-4). A Hewlett Packard Model 1084A with refractive index detector was used. The sugars were separated by a 20 cm x 4.6 mm I.D. Lichrosorb-NH₂ (5µm) column, using water/acetonitrile: 20/80 (v/v) as mobile phase. Flow rate of the solvents was 2.5 mL/min.

- 5 -

pH

pH in fermentation media was measured by a Radiometer pH meter.

STAFF

This study was undertaken at B.C. Research by Mr. E.G-H. Lee, microbiologist; Mr. T. Vo and Mr. L. Chung, analysts.

RESULTS

Fermentability of organosolv spent liquor (OSL) was evaluated at various concentrations added to synthetic medium. The specific concentrations of untreated and hydrolyzed OSL tested were 0.1, 1, 5, 10 and 25%. Growth and fermentation yields were evaluated after 3 days of incubation.

All three cultures grew well and produced solvents at 5% OSL (Tables 1 to 4). Only two cultures, S. cerevisiae and P. tannophilus grew and produced ethanol on media containing up to 10% OSL (Tables 1-3). The growth and solvent production by Cl. acetobutylicum were suppressed at 10% OSL (Table 1 and 4). None of the tested organisms grew or produced solvents on any of the media containing 25% or more OSL in the synthetic media (Tables 1-4).

Further hydrolysis of OSL by autoclaving OSL in 1N H₂SO₄ at 15 psi for 1 h did not enhance fermentability of OSL (Appendices I-b to III-b).

- 6 -

The results confirmed that inhibitory or toxic substances are present in the OSL as reported previously (5). Dilution of OSL to less than 10% is necessary to make fermentation possible.



E.G.-H. Lee
Principal Investigator
Fisheries and Biotechnology

J.C. Mueller
Head, Fisheries and Biotechnology

- 7 -

REFERENCES

1. Wentz, F.E., Marcy, A.D., and M.J. Gray. 1982. Analysis of wood sugars in pulp and paper industry samples by HPLC. J. Chromatogr. Sci. 20:349-352.
2. Paice, M.G., Jurasek, L. and M. Desrochers. 1982. Simplified analysis of wood sugars. Tappi 65(7): 103-106.
3. Götz, H. 1982. Analysis of Carbohydrates in Food in HPLC. Hewlett-Packard Application Note AN 232-14.
4. Tweeten, T.N. and C.B. Euston. 1980. Application of high performance liquid chromatography in the food industry. Food Technology 12: 29-37.
5. Lee, E.G-H. and J.C. Mueller. 1985. Evaluation of Hemicellulose Fractions for Fermentability - Phase I. Report submitted to Econotech Services Ltd. in May.

TABLE 1
Fermentability of Organosolv Spent Liquor (OSL)

Substrate	OSL Concentration (%)	Cultures	Growth*	Solvent Production*
Glucose or xylose (control synthetic medium)	n/a	<u>S. cerevisiae</u>	Yes	Yes
		<u>P. tannophilus</u>	Yes	Yes
		<u>Cl. acetobutylicum</u>	Yes	Yes
Organosolv Spent Liquor + synthetic medium	0.1%	<u>S. cerevisiae</u>	Yes	Yes
		<u>P. tannophilus</u>	Yes	Yes
		<u>Cl. acetobutylicum</u>	Yes	Yes
	1.0%	<u>S. cerevisiae</u>	Yes	Yes
		<u>P. tannophilus</u>	Yes	Yes
		<u>Cl. acetobutylicum</u>	Yes	Yes
	5%	<u>S. cerevisiae</u>	Yes	Yes
		<u>P. tannophilus</u>	Yes	Yes
		<u>Cl. acetobutylicum</u>	Yes	Yes
	10%	<u>S. cerevisiae</u>	Yes	Yes
		<u>P. tannophilus</u>	Yes	Yes
		<u>Cl. acetobutylicum</u>	Yes	No
	25%	<u>S. cerevisiae</u>	No	No
		<u>P. tannophilus</u>	No	No
		<u>Cl. acetobutylicum</u>	No	No

* Appendices I(a) to III(b)

TABLE 2

Ethanol Yields By *S. cerevisiae* Grown on
Organosolv Spent Liquor (OSL)

O.S.L. Added (%)	Glucose Used* (g/L)	Ethanol Produced* (g/L)	Ethanol Yields** (%)
None (control)	20.1	8.3	41.3
0.1	20.5	7.8	38.0
1.0	20.6	8.6	41.7
5.0	20.7	8.8	42.5
10.0	20.4	8.0	39.2
25.0	0	0	0

* Appendix I(a)

** % Ethanol yields = $\frac{\text{Ethanol produced g/L}}{\text{Glucose used (g/L)}} \times 100$

TABLE 3

Ethanol Yields By *P. tannophilus* Grown on
Organosolv Spent Liquor (OSL)

O.S.L. Added (%)	Xylose Used* (g/L)	Ethanol Produced* (g/L)	Ethanol Yields** (%)
None (control)	9.9	1.2	12.1
0.1	8.5	1.5	17.6
1.0	8.7	1.4	16.1
5.0	9.8	1.8	18.4
10.0	10.7	1.9	17.8
25.0	0	0	0

* Appendix II(a)

** % Ethanol yields = $\frac{\text{Ethanol produced (g/L)} \times 100}{\text{Xylose used (g/L)}}$

TABLE 4

Solvent Yields By *Cl. acetobutylicum* Grown on
Organosolv Spent Liquor (OSL)

O.S.L. Added (%)	Glucose Used* (g/L)	Solvent Produced** (g/L)	Solvent Yields*** (%)
None (control)	20.3	0.9	4.4
0.1	20.4	0.6	2.9
1.0	20.5	0.6	2.9
5.0	20.8	0.5	2.4
10.0	1.2	0	0
25.0	0	0	0

* Appendix III(a)

** Solvent produced = the sum of butanol, acetone, and ethanol

*** % Solvent yields = $\frac{\text{Solvent produced (g/L)} \times 100}{\text{Glucose used (g/L)}}$

I(a) Fermentability of Untreated Organosolv Spent Liquor (OSL)
By *Saccharomyces cerevisiae*

Fermentation Conditions: - 1% inoculum.
- batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
- stationary at 28°C.
- 0.1 to 25% OSL from cook #5 were added to synthetic medium.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Glucose (g/L)	Ethanol (g/L)
Glucose, yeast extract, and peptone (control medium)	start	1	-	6.1	0.02	20.1	0
		2	-	6.1	0.02	20.1	0
		Average	-	6.1	0.02	20.1	0
	72	1	+	2.9	2.5	0	8.1
		2	+	2.8	2.4	0	8.5
		Average	+	2.9	2.5	0	8.3
0.1% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.3	0
		2	-	6.1	0.02	20.7	0
		Average	-	6.1	0.02	20.5	0
	72	1	+	2.9	3.1	0	7.4
		2	+	2.6	3.5	0	8.1
		Average	+	2.8	3.3	0	7.8
1.0% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.4	0
		2	-	6.1	0.02	20.8	0
		Average	-	6.1	0.02	20.6	0
	72	1	+	2.7	2.8	0	8.7
		2	+	2.6	3.5	0	8.1
		Average	+	2.8	3.3	0	7.8
5% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.8	0.1
		2	-	6.1	0.02	20.6	0.1
		Average	-	6.1	0.02	20.7	0.1
	72	1	+	2.9	2.7	0	8.9
		2	+	3.1	3.4	0	8.6
		Average	+	3.0	3.1	0	8.8
10% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.3	0.2
		2	-	6.1	0.02	20.5	0.2
		Average	-	6.1	0.02	20.4	0.2
	72	1	+	2.9	2.9	0	7.9
		2	+	2.7	2.8	0	8.1
		Average	+	2.8	2.9	0	8.0
25% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.3	0.5
		2	-	6.1	0.02	20.8	0.6
		Average	-	6.1	0.02	20.6	0.6
	72	1	-	6.1	-	-	-
		2	-	6.1	-	-	-
		Average	-	6.1	-	-	-

I(b) Fermentability of Hydrolyzed Organosolv Spent Liquor (OSL)
By *Saccharomyces cerevisiae*

Fermentation Conditions: - 1% inoculum.
- batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
- stationary at 28°C.
- 0.1 to 25% OSL from cook #5 were added to synthetic medium.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Glucose (g/L)	Ethanol (g/L)
Glucose, yeast extract, and peptone (control medium)	start	1	-	6.1	0.02	20.1	0
		2	-	6.1	0.02	20.1	0
		Average	-	6.1	0.02	20.1	0
	72	1	+	2.9	2.5	0	8.1
		2	+	2.8	2.4	0	8.5
		Average	+	2.9	2.5	0	8.3
0.1% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	20.7	0
		2	-	6.1	0.02	20.8	0
		Average	-	6.1	0.02	20.8	0
	72	1	+	2.7	2.9	0	-
		2	+	2.8	2.6	0	-
		Average	+	2.8	2.8	0	-
1.0% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	22.6	0
		2	-	6.1	0.02	21.9	0
		Average	-	6.1	0.02	22.3	0
	72	1	+	2.8	2.6	0	-
		2	+	2.7	3.0	0	-
		Average	+	2.8	2.8	0	-
5% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	22.6	0.1
		2	-	6.1	0.02	21.9	0.1
		Average	-	6.1	0.02	22.3	0.1
	72	1	+	2.8	2.6	0	-
		2	+	2.7	3.0	0	-
		Average	+	2.8	2.8	0	-
10% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	23.1	0
		2	-	6.1	0.02	22.9	0
		Average	-	6.1	0.02	23.0	0
	72	1	+	3.2	2.2	1.3	-
		2	+	3.0	1.9	1.8	-
		Average	+	3.1	2.1	1.6	-
25% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	22.7	0.1
		2	-	6.1	0.02	22.5	0.1
		Average	-	6.1	0.02	22.6	0.1
	72	1	-	-	-	-	-
		2	-	-	-	-	-
		Average	-	-	-	-	-

II(a) Fermentability of Untreated Organosolv Spent Liquor (OSL)
By *Pachysolen tannophilus*

Fermentation Conditions:

- 1% inoculum.
- batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
- stationary at 28°C.
- 0.1 to 25% OSL from cook #5 were added to synthetic medium.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Xylose (g/L)	Ethanol (g/L)
Xylose, yeast extract, and peptone (control medium)	start	1	-	6.1	0.02	20.1	0
		2	-	6.1	0.02	20.3	0
		Average	-	6.1	0.02	20.2	0
	72	1	+	3.1	1.2	10.9	1.1
		2	+	2.6	1.1	9.6	1.2
		Average	+	2.9	1.2	10.3	1.2
0.1% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.2	0
		2	-	6.1	0.02	20.5	0
		Average	-	6.1	0.02	20.4	0
	72	1	+	2.9	1.5	12.6	1.4
		2	+	3.0	1.1	11.2	1.6
		Average	+	3.0	1.3	11.9	1.5
1.0% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.1	0
		2	-	6.1	0.02	20.4	0
		Average	-	6.1	0.02	20.3	0
	72	1	+	2.9	1.1	12.5	1.2
		2	+	3.2	1.5	10.7	1.5
		Average	+	3.1	1.3	11.6	1.4
5% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.2	0.1
		2	-	6.1	0.02	20.4	0.1
		Average	-	6.1	0.02	20.3	0.1
	72	1	+	3.0	1.5	11.2	1.8
		2	+	2.9	1.3	9.8	1.7
		Average	+	3.0	1.4	10.5	1.8
10% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.5	0.2
		2	-	6.1	0.02	20.4	0.2
		Average	-	6.1	0.02	20.5	0.2
	72	1	+	2.9	1.7	8.9	1.9
		2	+	2.8	1.4	10.7	1.8
		Average	+	2.9	1.6	9.8	1.9
25% untreated OSL + synthetic medium	start	1	-	6.1	0.02	20.3	0.5
		2	-	6.1	0.02	20.4	0.6
		Average	-	6.1	0.02	20.4	0.6
	72	1	-	6.1	-	-	-
		2	-	6.1	-	-	-
		Average	-	6.1	-	-	-

**II(b) Fermentability of Hydrolyzed Organosolv Spent Liquor (OSL)
By *Pachysolen tannophilus***

- Fermentation Conditions:**
- 1% inoculum.
 - batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
 - stationary at 28°C.
 - 0.1 to 25% OSL from cook #5 were added to synthetic medium.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Xylose (g/L)	Ethanol (g/L)
Xylose, yeast extract, and peptone (control medium)	start	1	-	6.1	0.02	20.1	0
		2	-	6.1	0.02	20.3	0
		Average	-	6.1	0.02	20.2	0
	72	1	+	3.1	1.2	10.9	1.1
		2	+	2.6	1.1	9.6	1.2
		Average	+	2.9	1.2	10.3	1.2
0.1% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	20.9	0
		2	-	6.1	0.02	20.5	0
		Average	-	6.1	0.02	20.7	0
	72	1	+	2.8	1.4	-	-
		2	+	2.6	1.1	-	-
		Average	+	2.7	1.3	-	-
1.0% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	20.9	0
		2	-	6.1	0.02	20.5	0
		Average	-	6.1	0.02	20.7	0
	72	1	+	2.8	1.4	-	-
		2	+	2.6	1.1	-	-
		Average	+	2.7	1.3	-	-
5% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	20.8	0
		2	-	6.1	0.02	21.1	0
		Average	-	6.1	0.02	21.0	0
	72	1	+	2.9	1.3	-	-
		2	+	3.3	1.1	-	-
		Average	+	3.1	1.2	-	-
10% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	21.7	0
		2	-	6.1	0.02	21.9	0
		Average	-	6.1	0.02	21.8	0
	72	1	+	2.9	1.2	-	-
		2	+	3.1	1.0	-	-
		Average	+	3.0	1.1	-	-
25% hydrolyzed OSL + synthetic medium	start	1	-	6.1	0.02	21.9	0.1
		2	-	6.1	0.02	21.8	0.1
		Average	-	6.1	0.02	21.9	0.1
	72	1	-	-	-	-	-
		2	-	-	-	-	-
		Average	-	-	-	-	-

III(a) Fermentability of Untreated Organosolv Spent Liquor (OSL)
By *Clostridium acetobutylicum*

Fermentation Conditions: - 1% inoculum.
- batch fermentation; (10 mL medium in a test tube containing invert "gas
vial").
- stationary at 28°C.
- 0.1 to 25% OSL from cook #5 were added to synthetic medium.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Glucose (g/L)	Butanol (g/L)	Acetone (g/L)	Ethanol (g/L)
Glucose, yeast extract, and peptone (control medium)	start	1	-	6.6	0.02	20.4	0	0	0
		2	-	6.6	0.02	20.2	0	0	0
		Average	-	6.6	0.02	20.3	0	0	0
	72	1	+	3.5	2.4	0	0.5	0.2	0.1
		2	+	3.8	2.3	0	0.6	0.2	0.1
		Average	+	3.7	2.4	0	0.6	0.2	0.1
0.1% untreated OSL + synthetic medium	start	1	-	6.6	0.02	20.5	0	0	0
		2	-	6.6	0.02	20.2	0	0	0
		Average	-	6.6	0.02	20.4	0	0	0
	72	1	+	3.5	2.5	0	0.2	0.1	0.1
		2	+	3.6	2.1	0	0.4	0.3	0.1
		Average	+	3.6	2.3	0	0.3	0.2	0.1
1.0% untreated OSL + synthetic medium	start	1	-	6.6	0.02	20.4	0	0	0
		2	-	6.6	0.02	20.5	0	0	0
		Average	-	6.6	0.02	20.5	0	0	0
	72	1	+	3.5	2.8	0	0.3	0.2	0.1
		2	+	3.1	2.5	0	0.3	0.2	0.1
		Average	+	3.3	2.7	0	0.3	0.2	0.1
5% untreated OSL + synthetic medium	start	1	-	6.6	0.02	20.4	0	0	0
		2	-	6.6	0.02	20.5	0	0	0
		Average	-	6.6	0.02	20.5	0	0	0
	72	1	+	3.5	2.8	0	0.2	0.2	0.1
		2	+	3.1	2.5	0	0.2	0.2	0.1
		Average	+	3.3	2.7	0	0.2	0.2	0.1
10% untreated OSL + synthetic medium	start	1	-	6.6	0.02	20.9	0	0	0.2
		2	-	6.6	0.02	20.8	0	0	0.2
		Average	-	6.6	0.02	20.9	0	0	0.2
	72	1	+	4.9	0.6	20.1	0	0	0
		2	+	5.2	0.7	19.2	0	0	0
		Average	+	5.1	0.7	19.7	0	0	0
25% untreated OSL + synthetic medium	start	1	-	6.6	0.02	20.6	0	0	0.2
		2	-	6.6	0.02	20.7	0	0	0.2
		Average	-	6.6	0.02	20.7	0	0	0.2
	72	1	-	6.6	-	-	-	-	-
		2	-	6.6	-	-	-	-	-
		Average	-	6.6	-	-	-	-	-

III(b) Fermentability of Hydrolyzed Organosolv Spent Liquor (OSL)
By *Clostridium acetobutylicum*

Fermentation Conditions: - 1% inoculum.
- batch fermentation; (10 mL medium in a test tube containing invert "gas vial").
- stationary at 28°C.
- 0.1 to 25% OSL from cook #5 were added to synthetic medium.

Type of Substrate	Incubation Time (h)	Run #	Gas Produced	pH	Biomass (g/L)	Glucose (g/L)	Butanol (g/L)	Acetone (g/L)	Ethanol (g/L)
Glucose, yeast extract, and peptone (control medium)	start	1	-	6.6	0.02	20.4	0	0	0
		2	-	6.6	0.02	20.2	0	0	0
		Average	-	6.6	0.02	20.3	0	0	0
	72	1	+	3.5	2.4	0	0.5	0.2	0.1
		2	+	3.8	2.3	0	0.6	0.2	0.1
		Average	+	3.7	2.4	0	0.6	0.2	0.1
0.1% hydrolyzed OSL + synthetic medium	start	1	-	6.6	0.02	20.7	0	0	0
		2	-	6.6	0.02	20.8	0	0	0
		Average	-	6.6	0.02	20.8	0	0	0
	72	1	+	3.9	2.1	0	-	-	-
		2	+	3.6	2.6	0	-	-	-
		Average	+	3.8	2.4	0	-	-	-
1.0% hydrolyzed OSL + synthetic medium	start	1	-	6.6	0.02	21.8	0	0	0
		2	-	6.6	0.02	21.5	0	0	0
		Average	-	6.6	0.02	21.7	0	0	0
	72	1	+	3.5	2.7	0	-	-	-
		2	+	3.8	2.6	0	-	-	-
		Average	+	3.7	2.7	0	-	-	-
5% hydrolyzed OSL + synthetic medium	start	1	-	6.6	0.02	22.6	0	0	0
		2	-	6.6	0.02	21.9	0	0	0
		Average	-	6.6	0.02	22.3	0	0	0
	72	1	-	3.8	2.8	0.7	-	-	-
		2	-	3.3	2.3	0.5	-	-	-
		Average	-	3.6	2.6	0.6	-	-	-
10% hydrolyzed OSL + synthetic medium	start	1	-	6.6	0.02	23.1	0	0	0
		2	-	6.6	0.02	22.5	0	0	0
		Average	-	6.6	0.02	22.6	0	0	0
	72	1	+	6.5	-	-	-	-	-
		2	+	6.6	-	-	-	-	-
		Average	+	6.6	-	-	-	-	-
25% hydrolyzed OSL + synthetic medium	start	1	-	6.6	0.02	22.7	0	0	0.1
		2	-	6.6	0.02	22.5	0	0	0.1
		Average	-	6.6	0.02	22.6	0	0	0.1
	72	1	-	6.5	-	-	-	-	-
		2	-	6.6	-	-	-	-	-
		Average	-	6.6	-	-	-	-	-